BIOMETRICAL GENETICS

By the same Author

THE MEASUREMENT OF LINKAGE
IN HEREDITY

STATISTICAL ANALYSIS IN BIOLOGY

THE ELEMENTS OF GENETICS
(with C. D. DARLINGTON)

BIOMETRICAL GENETICS

THE STUDY OF CONTINUOUS VARIATION

BY

K. MATHER

D.Sc., Ph.D.

Head of the Genetics Department

John Innes Horticultural Institution, London

WITH SIXTEEN DIAGRAMS

DOVER PUBLICATIONS, INC.

Printed and bound in the United States of America

To

R. A. FISHER

Statistician and Geneticist

PREFACE

THE PROPERTIES of continuous variation are basic to the theory of evolution and to the practice of plant and animal improvement. Yet the genetical study of continuous variation has lagged far behind that of discontinuous variation.

The reason for this situation is basically methodological. Mendel gave us not merely his principles of heredity, but also a method of experiment by which these principles could be tested over a wider range of living species, and extended into the elaborate genetical theory of today. The power of this tool is well attested by the speed with which genetics has grown. In less than fifty years, it has not only developed a theoretical structure which is unique in the biological sciences, but has established a union with nuclear cytology so close that the two have become virtually a single science offering us a new approach to problems so diverse as those of evolution, development, disease, cellular chemistry and human welfare. Much of this progress would have been impossible and all would have been slower without the Mendelian method of recognizing and using unit differences in the genetic materials.

These great achievements should not, however, blind us to the limitations inherent in the method itself. It depends for its success on the ability to assign the individuals to classes whose clear phenotypic distinctions reveal the underlying genetic differences. A certain amount of overlap of the phenotypic classes can be accommodated by the use of genetical devices; but where the variation in phenotype is fully continuous in its frequency distribution, so that no such classes can be defined, the method cannot be used. A different approach is required, one based on the use of measurement rather than frequency.

The first steps were taken nearly forty years ago, when the theory of cumulative factors or multifactorial inheritance, as it was variously called, was formulated. The full implications of this theory have, however, only gradually become realized. In the same way the special types of experiment and statistical analysis necessary for the study of continuous variation have only gradu-

ally become available. Nevertheless, though slow, progress has been real and we are now in a position to see not merely how continuous variation can be explained genetically, but also how experiments can be conducted enabling us to understand and to measure the special genetical quantities in terms of which continuous variation can be analysed and its behaviour in some measure predicted.

The present book does not aim at covering the whole literature of the subject. I have concentrated attention rather on trying to show the kind of evidence upon which the genetical theory of continuous variation is based, to bring out the special problems which it raises, to see how the familiar genetical concepts must be adapted to their new use, and to outline an analytical approach which can help us to understand our experimental results, particularly those which can be obtained from plant material. In doing so I have assumed some knowledge of genetics and statistics. To have done otherwise would have made the text unnecessarily long, for this information can be gained from a variety of other sources.

The data with which I have had to work have been limited by the paucity of experiments adequate in both scope and description and I have therefore been unable to try out the methods, which are described, in as wide a variety of circumstances as could have been wished. These methods are in no sense exclusive or final; indeed their limitations require no stressing. But improvements can be brought about only as more and better experiments are undertaken ; and such experiments cannot be planned until we have explored the scope and limitations of those we already have. Improvement of experiment, refinement of analysis and development of theory must be simultaneous and progressive.

Among the experiments upon which I have been able to draw none has been more informative than that on ear conformation in barley, hitherto unpublished. This experiment was made in collaboration with Dr. Ursula Philip, now of the Department of Zoology, King's College, Newcastle-on-Tyne, and I wish to express my indebtedness to her for allowing its results to be published in this way.

K. M.

April 1947

CONTENTS

CHAPTER		PAGE
1	THE GENETICAL FOUNDATION	
	1. Biometry and Genetics	1
	2. Polygenic Segregation and Linkage	5
	3. Polygenes and Major Genes	15
2	CHARACTERS	
	4. Phenotype and Genotype	23
	5. Genetic Analysis and Somatic Analysis	26
	6.* The Combination of Characters	31
3	SCALES	
	7. The Principles of Scaling	38
	8. The Criteria of Scaling	40
	9. Some Examples of Scaling Tests	44
	10. Transformation and Residual Interaction	49
4	THE COMPONENTS OF VARIATION	
	11. Dominance and Potence	52
	12. Variation in F_2 and F_3	54
	13. Partitioning the Variation in F_2 and its Derivatives	58
	14. Backcrosses	68
	15. Randomly Breeding Populations	72
5	LINKAGE	
	16. The Effect of Linkage	82
	17. The Test of Linkage	92
	18.* Coupling in Barley	97
6	THE NUMBER OF EFFECTIVE FACTORS	
	19. The Sources of Estimates	102
	20. The Process of Estimation	108
	21.* Prediction in the Presence of Linkage	115
	22. The Speed of Advance under Selection	119
7	RESULTS AND CONCEPTS	
	23. Experiments in Biometrical Genetics	126
	24. Dominance and Interaction	136
	25. The Effective Factor	143
	GLOSSARY OF SYMBOLS	153
	REFERENCES	156
	INDEX	159

* In collaboration with Dr. U. Philip.

CHAPTER 1

THE GENETICAL FOUNDATION

1. BIOMETRY AND GENETICS

THE GROWTH of genetical science as we know it today began with the re-discovery of Mendel's work in 1900. Nevertheless, at the time of that event there were already genetical investigations in active progress; investigations which, although contributing relatively little to the development of genetical theory, still have an importance of their own. These were begun by Francis Galton, who published a general account of his methods and findings in *Natural Inheritance* (1889), and were continued by Karl Pearson and his pupils. From them the application of statistical mathematics to biological problems received a great impetus, and if for this reason alone they mark a significant step in the growth of quantitative biology.

The relative failure of this work in its avowed purpose, that of elucidating the relations of parent to offspring in heredity, stems from a variety of causes. Mendel himself regarded the failure of his predecessors as due to their experiments not making it possible 'to determine the number of different forms under which the offspring of hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their statistical relations'. While Galton's work can hardly be regarded as failing in the third respect, the nature of the material he chose rendered it impossible for him to succeed in the other two. His extensive use of human data, with its small families and genetically uncertain ancestries introduced difficulties enough; but it was the choice of metrical or quantitative characters, like stature in man, that foredoomed the work from the point of view of the laws of inheritance. These characters show continuous gradations of expression between wide extremes, the middle expression being most common in any family or population, and the frequency of occurrence falling away as we proceed towards either extreme (see Fig. 2). The distributions of frequencies of the various grades of expression sometimes, as with stature in man, approximate closely to the Normal curve; but while retaining the same general shape they depart in other cases from this precise form, for example by being asymmetrical. The simple Mendelian

ratios, with their clear implication of the particulate or discontinuous nature of hereditary constitution and transmission, depend on the use of characters by which individuals could be classed unambiguously into a few (usually two) distinct groups: they cannot come from continuous variation. Indeed, Mendel himself deliberately neglected such variation in his material, presumably with the clear recognition that it could only have a distracting influence in his analyses.

Yet this continuous variation could not be completely overlooked. Darwin himself had emphasized the importance of small cumulative steps in evolutionary change, and observation on any living species, especially the most familiar of all, man, showed how much of the variation between individuals was of this kind. The genetical problem of continuous variation remained therefore a challenge to geneticists; the more so as biometrically Galton and Pearson had clearly shown such variation to be at least in part hereditable, even although they had failed to discover the mode of transmission. Neither the Galtonian nor the Mendelian method was of itself capable of supplying the solution. The understanding of continuous variation awaited a fusion of the two methods of approach, the genetical and the biometrical, for each supplied what the other lacked. The one gave us the principles on which the analysis must be based; the other showed the way in which to handle continuous variation, the way of representing it in a form which made fruitful analysis possible.

Fusion was, however, delayed by a rivalry which arose between Biometricians and Mendelians as soon as Mendel's work was rediscovered. This was aggravated by divergent opinions on the importance of continuous and discontinuous variation in evolutionary change, and acerbated by the polemics of the protagonists. In time, attempts to reconcile the two views became welcome to neither party. The original discordance seems to have arisen because neither side understood the full implications of Mendel's fundamental separation of determinant and effect, of genotype and phenotype. The biometricians seem to have regarded continuous somatic variation as implying continuous genetic variation, and the Mendelians seem to have considered discontinuous genetic variation as incompatible with anything but obviously discontinuous somatic variation. Indeed, de Vries took continuity of variation in the phenotype as a criterion of its non-heritability.

Two important steps had to be taken, therefore, before the biometrical and genetical methods could be brought together. In 1909

Johannsen published his *Elemente der exakten Erblichkeitslehre*. In it he described the experiments with beans which led him to formulate his pure line theory. In particular he showed that heritable and non-heritable agencies were jointly responsible for the variation in seed weight with which he was concerned; that their effects were of the same order of magnitude; and that there was no means, other than the breeding test, of distinguishing between their contributions to the variation. The relations between genotype and phenotype were thus becoming clearer. The effects of discontinuity of the genotype could be smoothed out and continuous variation realized in the phenotype by the action of the environment.

In the same year a second Scandinavian geneticist, Nilsson-Ehle, took the other step. He found that in wheat and oats there existed hereditary factors whose actions were very similar, if not exactly alike. There were three such factors for red *versus* white grain in wheat. Any one of them, when segregating alone, gave an F_2 ratio of 3 red : 1 white. Two of them segregating together gave 15 : 1 for red : white, and all three gave a 63 : 1 ratio. That the red-grained plants in these F_2's were of various genetical constitutions could be shown by growing F_3 families. Some of these gave 3 red : 1 white, others 15 : 1, others 63 : 1 and still others all red. Yet there were no detectable differences in colour between plants owing their redness to the different factors. There were certainly some differences in redness, but these appeared to be associated more with the number of factors, than with the particular factors, present. The first degree of redness would be given equally by the three genotypes **Aabbcc**, **aaBbcc** and **aabbCc**; the second by the six genotypes **AAbbcc**, **aaBBcc**, **aabbCC**, **AaBbcc**, **AabbCc** and **aaBbCc**; and so on. It thus appeared that different factors could have similar actions, and actions which were, at least in some measure, cumulative.

These factors in wheat and oats had effects sufficiently large for Mendelian analysis to be possible; but it was realized by Nilsson-Ehle, and also independently by East, that similar factors of smaller individual action could account for continuous quantitative variation if enough of them were segregating. Each factor would be inherited in the Mendelian way, and its changes would be discontinuous or qualitative. Yet with a number of such factors, having similar and cumulative action, many different dosages would be possible, of which the intermediate ones would be the most common (Fig. 1). With phenotypic expression proportional

to factor dosage, variation would be quantitative, would follow Galton's frequency curves and would be nearly continuous. Continuity would be completed by the blurring effect of non-heritable

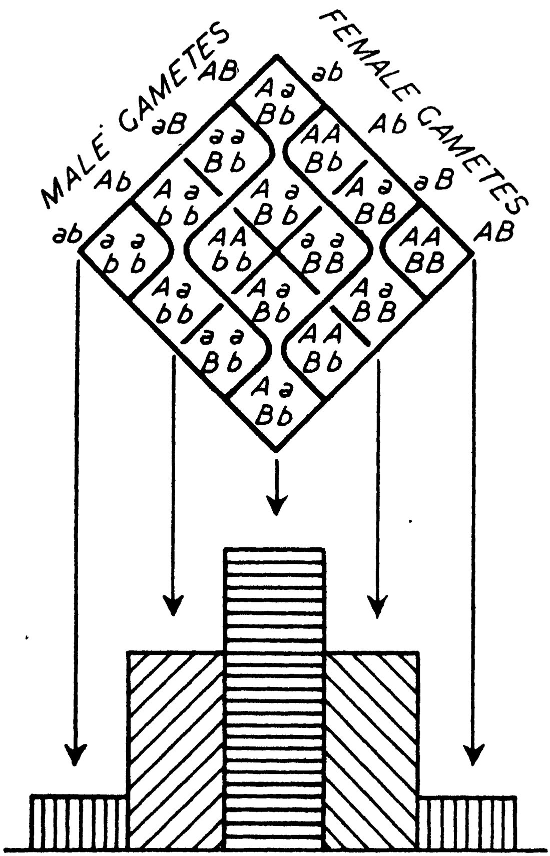


FIG. 1

The polygenic or multiple factor theory. The distribution of phenotypes obtained in a F_2 with two genes of equal and additive effect but without dominance, neglecting non-heritable variation. The phenotypic expression is proportional to the number of capital letters in the genotype There would be seven phenotypic classes with three such genes, nine classes with four genes, and $2n+1$ classes with n genes

agencies, which would of course make the phenotypic ranges of the various genotypes overlap.

During the next ten years this multiple factor hypothesis, as it was called, was applied to data from a variety of organisms, notably by East and his collaborators, and by Fisher. The former

showed that the inheritance of a number of continuously variable characters in tobacco and maize could be fully accounted for on this view (e.g. East 1915, Emerson and East 1913). Fisher carried the integration of biometry and genetics still further. He demonstrated that the results of the biometricians themselves, particularly the correlations which they had found between human relatives, must follow on the new view (Fisher 1918). From the biometricians' own data he was able to produce evidence of dominance of the multiple factors, and he attempted the first partition of continuous variation into the components which the multiple factor hypothesis led him to expect.

2. POLYGENIC SEGREGATION AND LINKAGE

The essential features of the multiple factor hypothesis are two: that the governing factors or genes are inherited in the Mendelian fashion; and that they have effects similar to one another, supplementing each other and small in relation to the non-heritable variation, or at least in relation to the total variation. In this way smooth, continuous variation of the phenotype could arise from discontinuous, quantal variation of the genotype.

There is an obvious danger in postulating these multifactorial or polygenic systems. The constituent genes are so alike in their effects and so readily mimicked by non-heritable agencies, that they cannot be identified individually within the systems. Since such genes obviously cannot be followed by the Mendelian technique, how may we be sure that they are in truth borne on the chromosomes and so subject to Mendelian inheritance?

On the negative side there is the evidence of reciprocal crosses. Though these sometimes differ a little in respect of continuously variable characters, presumed to be under polygenic control, they do so no more often than is the case with discontinuously variable characters. The two parents therefore generally contribute equally to the genotype of the offspring in the way expected of nuclear heredity, and not unequally as would be expected if inheritance was of some other kind.

More positive evidence is, however, available. The properties characteristic of nuclear borne genes are two, viz. segregation and linkage. Although neither segregation nor linkage of the genes under discussion can be observed by the usual methods, the necessary tests can been made in other ways.

If we take two different inbred, and therefore very nearly true-breeding strains, both they and their F_1 will show variation

virtually only in so far as non-heritable agencies are at work. But genetical segregation of the nuclear genes which differentiate the parents will occur in F_2, and the heritable variation to which it leads will be added to the non-heritable. The F_2 should therefore

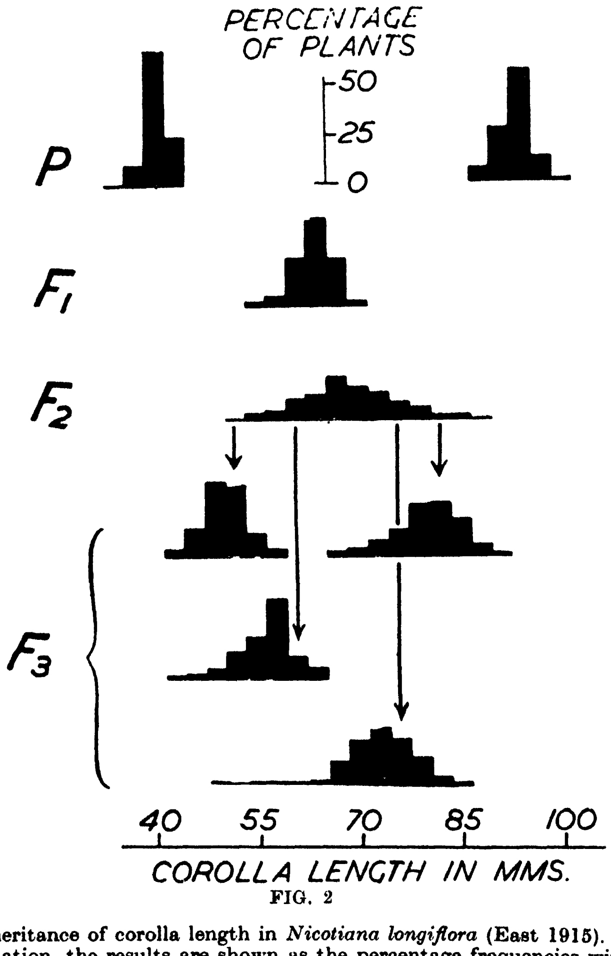


FIG. 2

The inheritance of corolla length in *Nicotiana longiflora* (East 1915). For ease of presentation, the results are shown as the percentage frequencies with which individuals fall into classes, each covering a range of 3 mms. in corolla length and centred on 34, 37. 40, etc., mms. This grouping is quite artificial and the apparent discontinuities spurious: corolla length actually varies continuously.

The means of F_1 and F_2 are intermediate between those of the parents. The means of the four F_3 families are correlated with the corolla length of the F_2 plants from which they came, as indicated by the arrows. Variation in parents and F_1 is all non-heritable, and hence is less than that in F_2 which shows additional variation arising from the segregation of the genes concerned in the cross. Variation in F_3 is on the average less than that of F_2 but greater than that of parents and F_1. Its magnitude varies among the different F_3's, according to the number of genes which are segregating

be more variable than the parents and the F_1: its frequency distribution will be broader and flatter. Furthermore, as Mendel showed, each gene is homozygous in half the F_2 individuals. Segregation will still occur in F_3 families, but it will be for only half the genes on the average. The average variation of F_3 families will therefore lie between that of F_2, on the one hand, and parents and F_1 on the other; but the families will differ among themselves, some having variances approaching one extreme, some the other and most being intermediate. At the same time the homozygous genes by which the F_2 individuals differed will give rise to differences between the mean phenotypes of the F_3 families; and these means will be correlated with the phenotypes of the F_2 parents. Even where the parental strains are not nearly true-breeding, the F_2 will generally (though not inevitably) show greater variation than either F_1 or parents.

Thus the necessary test of segregation is to be found in the relative variation of the different generations following crossing. It is sufficient to say that whenever a critical test has been made, and many have been made, the results have accorded with the expectation based on nuclear inheritance. A characteristic case is shown in Fig. 2.

Tests of linkage, the second property of nuclear genes, may be of two kinds. We may seek for linkage of the quantitative genes (or polygenes if we name them after the polygenic variation they determine) with genes of major effect, capable of being followed by Mendelian methods. Or we may seek for linkage between polygenes themselves.

The first case of apparent linkage between polygenes and a major gene was reported by Sax (1923). He crossed a strain of *Phaseolus vulgaris*, having large coloured seeds, with another whose seeds were small and white. Seed size showed itself to be a continuously variable character, but pigmentation proved to be due to a single gene difference, the F_2 giving a ratio of 3 coloured- : 1 white-seeded plant. By means of F_3 progenies the coloured F_2 plants were further classified into homozygotes and heterozygotes. On weighing the beans from the three classes of F_2 plant, **PP**, **Pp** and **pp** (**P** giving pigment and **p** no pigment), the average bean weights shown in Table 1 were obtained.

The standard errors show the differences in seed weight to be significant. As in the parents, **P** is associated with large seeds and **p** with small ones. Indeed, the average weight is nearly proportional to the number of **P** allelomorphs present.

TABLE 1

Bean Weight (in Centigrams) in a *Phaseolus vulgaris* F_2
(Sax, 1923)

Number of Plants	Colour constitution	Average seed weight
45	**PP**	30·7 ±0·6
80	**Pp**	28·3 ±0·3
41	**pp**	26·4 ±0·5

This is not, of course, final evidence of linkage of one or more polygenes governing seed weight with the major gene governing pigmentation. The effect could be due to a pleiotropic secondary effect of **P** itself. Such a criticism has, however, been ruled out in other experiments. Rasmusson (1935) investigated the variation of flowering time in crosses of the garden pea. Flowering time was expressed as a deviation, in terms of days, from the average flowering time of certain standard varieties grown each year for this purpose. A positive deviation indicated later flowering, and a negative deviation earlier flowering than the standard.

To take one of his crosses as an example, the variety Gj with coloured flowers gave a mean flowering time of 8·5, while Bism with white flowers gave −9·3. Colour versus white in the flower depends on a single major gene, **A-a**. In the F_2 between these varieties the coloured plants had a mean flowering time of 5·37±0·31 and the whites 2·11±0·76. The difference is significant and the coloureds are later than the whites, as would be expected from the parents. The difference is smaller than that between the parents, but this only shows that the association between the pigmentation gene and the polygenic system governing flowering time is incomplete.

So far the results are like those in beans; but the cross had been made on an earlier occasion, and from it an early coloured strain (HRT-II) had been selected. Its mean flowering time was nearly as early as that of Bism, namely −6·1. In the F_2 of HRT-II × Bism, the coloureds gave a mean of −7·97±0·36 and the whites −8·30±0·81. The flowering-time difference associated with colour had vanished. Hence the difference in the original cross must have been due to one or more flowering-time genes linked with the major gene governing pigmentation. HRT-II contained the recombinant chromosome carrying the colour gene from Gj and early flowering gene or genes from Bism.

To round off the case, it may be observed that in a cross of

HRT-II with a late flowering white variety, St, the coloured plants of F_2 had a mean flowering time of $-1{\cdot}24 \pm 0{\cdot}20$ and the whites $1{\cdot}63 \pm 0{\cdot}23$. The relation is here reversed in the way to be expected from linkage.

TABLE 2

Flowering Time in Peas (Rasmusson, 1935)

Cross	Mean flowering time of F_2 plants		Flowering time difference (A−a)
	Coloured (A)	White (a)	
Gj × Bism	5·37	2·11	3·26
HRT-II × Bism	−7·97	−8·30	0·33
HRT-II × St	−1·24	1·63	−2·87

Many cases of linkage between major genes and polygenes controlling continuous variation have been reported, although in the majority of them the possibility of pleiotropic action of the major gene has not been finally excluded by the demonstration of recombination. In *Drosophila melanogaster*, where all the chromosomes can be marked by major genes, it has been possible to show that they all carry polygenes affecting a single continuously variable character, such as egg size (Warren 1924). In several of these experiments it has also been shown, by comparing a number of unmarked chromosomes with the same marked tester chromosome, that the differences cannot be due to the major gene itself. There must also be polygenes acting. The case described by Mather (1942) in this fly may be taken as an example.

The distribution among the chromosomes of the genes affecting the number of abdominal chaetae, a continuously variable character, was followed in four stocks, O, B, H and L. Each of the four was crossed to a common tester stock in which the large second and third chromosomes were marked by the genes **Pm** and **Sb** respectively. The small fourth chromosome was not marked and so could not be followed. The X chromosome was marked by the gene **B** in B and H themselves, and so could be traced in their test crosses; but it was unmarked and could not be followed in the test crosses with O and L. F_2's were raised from the four F_1's, which were also backcrossed to the parent O, B, H or L as the case might be. Recombination is of course absent from male flies. In the females it was largely suppressed for the second chromosomes by an inversion, and partly in the third chromosomes by

a smaller inversion, but was not affected in the X. The average chaeta numbers of flies in the various classes distinguished by the marker genes were counted, and the effects of the various chromosomes thereby assessed. The results from F_2's and backcrosses are pooled in Table 3.

TABLE 3

Number of Abdominal Chaetae in *Drosophila melanogaster* (Mather, 1942)

Stock	Mean chaeta number		Chaeta number differences ascribable to chromosomes*		
	♂	♀	X	II	III
O	39·9	44·6	—	0·93	−0·67
B	36·1	43·5	0·89	0·23	−0·96
H	44·4	52·3	3·10	3·09	0·48
L	27·6	34·5	—	−1·05	−2·65
Average					
O and B	38·0	44·1	—	0·58	−0·81
H „ L	36·0	43·4	—	1·02	−1·09

* Expressed in all cases as the excess over the tester.

Comparison	Difference in Chromosomes		
	X	II	III
O–B	—	0·70±0·91	0·29±0·85
O–H	—	−2·17±0·91	−1·15±0·85
O–L	—	1·98±0·85	1·98±0·85
B–H	−2·21±1·12	−2·86±0·98	−1·44±0·85
B–L	—	1·28±0·91	1·69±0·85
H–L	—	4·14±0·91	3·13±0·85

The excesses in action of the four tested second chromosomes over the common tester, as measured by average number of chaetae, range from −1·05 to 3·09, and those of the third chromosomes from −2·65 to 0·48. These results may also be expressed as comparisons of the excesses with one another, thereby eliminating the tester chromosomes and with them the effects of the major genes used as markers. Thus the excess of the second chromosome from O over that from the tester was 0·93. The excess of the second chromosome from B was 0·23. The chromosome from O therefore has an excess of 0·93–0·23 over that from B. The comparisons

arrived at in this way are shown in the lower part of Table 3, and it is clear from the standard errors that the polygenic contents of the second and third chromosomes from O, B, H and L must be regarded as differing amongst themselves. There is a similar difference between the X chromosomes of B and H, the only two in whose crosses this chromosome could be followed.

We may take the analysis still further. The lines B and H differed by 8·3 chaetae in males and by 8·8 in females. The differences associated with the three major chromosomes X, II and III proved to be 2·21, 2·86 and 1·44 respectively, giving a total of 6·51 chaetae. The test therefore accounts for three-quarters of the difference between the stocks themselves. Now the technique of raising F_2's and backcrosses would, in this case, reveal the full action of only those genes from O, B, H and L genes, which were fully recessive to their allelomorphs from the tester stock. Fully dominant polygenes from O, B, H and L would entirely escape detection, and the effects of partially dominant polygenes would be observed only in proportion to their recessiveness. The total of 6·5 chaetae accounted for by reference to the chromosomes cannot therefore represent the action of all the polygenes by which B and H differed, except in the unlikely event of them all being fully recessive to their allelomorphs from the tester. So far as we can judge from this test, therefore, not only is the continuous variation in chaeta number under the control of polygenes in the chromosomes, but all the heritable variation between the stocks can reasonably be ascribed to such polygenes.

This experiment is of interest in a different connection. Lines H and L were originally derived by selections for high and low chaeta numbers respectively from the cross between B and O. It is clear from the table that the genic contents of the various chromosomes have been changed in this process. Thus O and B do not differ significantly in either the second or the third chromosome, though O is slightly above B for each. But O is certainly lower than H and higher than L in both chromosomes, while H and L differ even more markedly.

Now the average excess of H and L over the tester is 1·02 for the second and −1·09 for the third chromosome. These values resemble those of 0·58 and −0·81 obtained when the excesses of O and B are averaged. It would appear that H and L between them contain the same polygenes affecting chaeta number as O and B, the process of selecting H and L from B × O having resulted mainly in a redistribution of these genes. New differences do not appear

to have arisen to any extent. This agrees with findings that mutation would be incapable of explaining any but a small part of the selective responses obtained in establishing the H and L lines (Mather, 1941). In other words, the polygenes affecting abdominal chaeta number must have been recombined in the selected lines, so that +– and –+ combinations within the chromosomes of O and B have been replaced by ++ and –– in H and L respectively. Not only do polygenes show linkage with major genes, they show linkage with one another. Both the types of linkage that we foresaw are therefore demonstrated in this experiment.

In such a selection experiment, where response depends almost entirely on the redistribution by recombination of the genes within chromosomes, continuously variable characters other than the one for whose expression selection is practised must often be affected. For wherever the genes controlling a second character are intermingled along the chromosomes with those controlling the operative character, recombination of the one set will mean recombination of the other. Fixation by selection of the redistributed gene

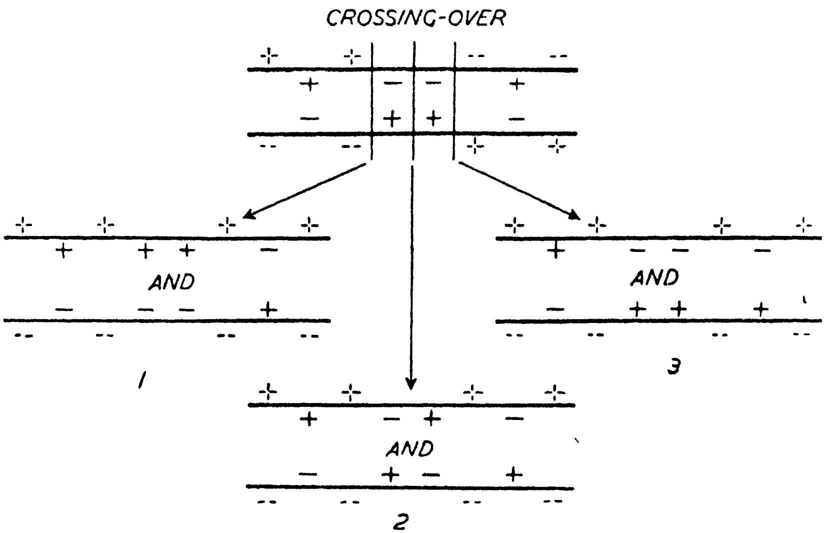


FIG. 3

Correlated responses to selection. Recombination of the genes controlling th primary character (-¦- and – –) is accompanied by recombination of the gene controlling the secondary character for which no selection is practised (+ and –) This recombination may lead to no unbalance (2) or to unbalance in either direction (1 and 3) of the genes controlling the secondary character according to the position of the crossing-over which gave rise to the recombination. The response of this character to selection for the primary one may therefore be in either direction or may be absent

combinations for the one character will then mean fixation of redistributed gene combinations for the other with the consequent possibility of a change in phenotype (Fig. 3). The second character can thus show a correlated response to a selection which did not aim at altering it, although the direction and magnitude of this correlated response may well be unpredictable (Wigan and Mather, 1942).

Such a correlated response must have occurred in selecting the H and L lines from B × O, for the fertility of the flies fell in both the selected lines. Falls in fertility are indeed a general accompaniment of selective changes in chaeta number in *Drosophila*. They have also been found in fishes (Svärdson, 1944). This effect might, of course, be ascribed to pleiotropic action of the polygenes if the relation of the two characters proved to be constant. Evidence is, however, now available against the assumption of pleiotropy (Mather and Harrison, 1948).

Selection was practised for increase in chaeta number on flies from a cross between two *Drosophila* stocks (Fig. 4). The mean number of chaetae rose for 20 generations and, as usual, fertility fell at the same time. After these 20 generations of selection the fertility was so low that the line could not be maintained under selection. Mass culture was then resorted to and the fertility immediately began to rise, doubtless as a result of natural selection for fertility within the culture bottles. This increase in fertility was accompanied by a correspondingly rapid fall in chaeta number, as indeed we should expect whether the effects on fertility and chaeta number were pleiotropically determined by the same genes or whether they were due to linkage of genes lowering fertility with those increasing chaeta number.

After three generations of mass culture selection was again practised for increased chaeta number. Four generations of such selection restored the chaeta number to the peak value originally obtained at the time when mass culture became necessary. But this time there was not the same loss of fertility, and the new high line maintained this peak value for over eighty generations after selection was again replaced by mass culture. Furthermore, not only did this new mass culture maintain itself at the level where fertility after the first selection had been so low, but it did so even though it had within itself all the genic materials for a fall in chaeta number, as was shown when a downward selection was successfully attempted with it later. Thus lower fertility was now associated, not with higher but with lower chaeta number.

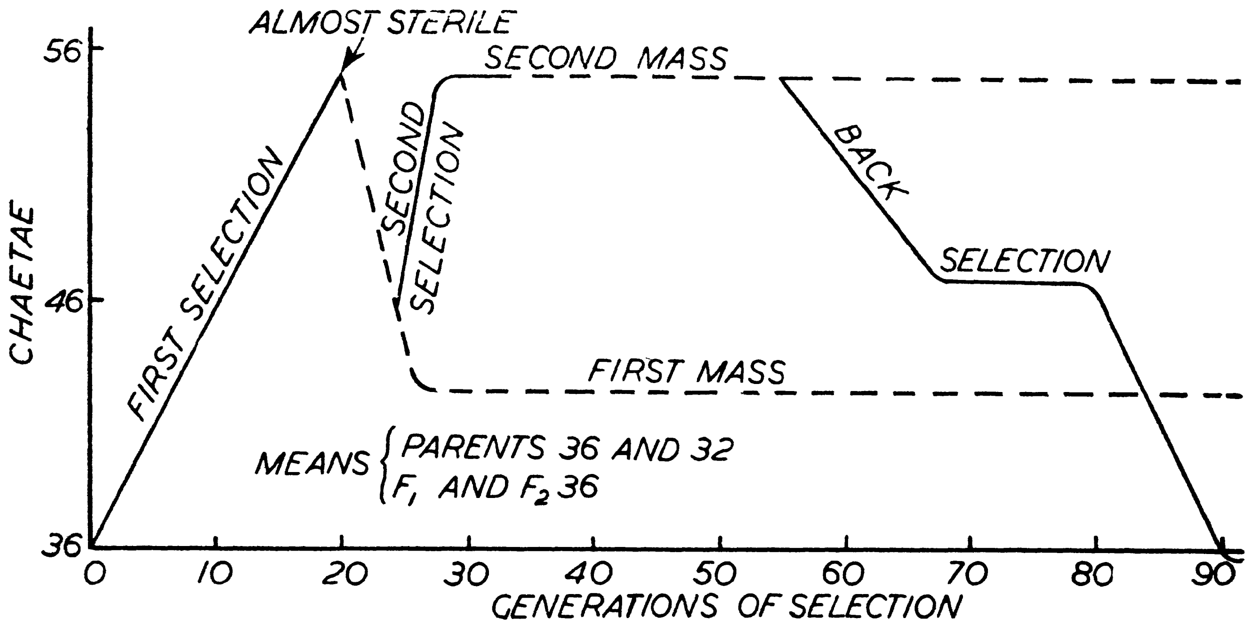


FIG. 4

Selection for increased number of abdominal chaetae in *Drosophila melanogaster* (Mather and Harrison, 1948) In the first selection, increase in mean chaeta number was accompanied by a decrease in fertility When selection for chaeta number was relaxed fertility took charge and chaeta number fell The second selection for chaeta number gave no correlated response in fertility, and the second relaxation of selection was followed by no fall in chaeta number, even though the stock was not homozygous as shown by successful back selection Solid lines indicate selection, and broken lines mass culture with selection

The conclusion is clear. In the second high selection the linkage of low fertility with higher chaeta number was broken, so that the second massed line had not the incubus of poor fertility combinations which had been present after the first selection. Such a reassociation must often follow by recombination on the assumption of linkage. It is capable of no simple explanation on the alternative assumption of pleiotropic action. It would thus appear that polygenes controlling different characters may be linked, as well as those controlling the same character.

Nor is this linkage confined to the polygenes controlling chaeta number and fertility. In the selection experiment which we have just described, selection for chaeta number was observed to produce correlated responses in number of spermathaecae, mating behaviour, and the frequency of appearance of certain eye abnormalities as well as in fertility. Other characters may also, of course, have been affected but have escaped notice. However this may be, the number of correlated responses actually observed gives an indication of the complexity of the linkage relations between the different polygenic systems.

We have now seen that the genes controlling continuous variation segregate in the same generations as do those major genes controlling discontinuous Mendelian variation; and that they show linkage with one another as well as with the major genes marking particular chromosomes. Furthermore, there is little evidence of differences in reciprocal crosses in the way expected if cytoplasmic inheritance were involved. And in the case where a partial balance sheet could be struck it accorded as well as could be expected with the view that all the heritable variation was accountable by genes carried on the chromosomes. The nucleus is apparently as potent relative to the remainder of the cell in its control of continuous variation as it is in its control of major discontinuities.

3. POLYGENES AND MAJOR GENES

The use of major genes as markers has allowed us not merely to see that the polygenes are borne on the chromosomes; it has enabled us to show that in *Drosophila* different members of the same polygenic system are carried on all three of the major chromosomes, X, second and third (Table 3). Each of these chromosomes gave evidence of change through recombination of the polygenes it carried, so that there must be at least six polygenes in the system governing continuous variation in the number of abdominal chaetae.

Now other crosses in *Drosophila* do not behave the same as the one described, in respect of this character (Mather, 1941), and we must therefore assume that they differ in other genes which are also members of this polygenic system affecting the number of abdominal chaetae. The number of polygenes in the system must thus exceed our minimum of six, although we cannot say by how much this minimum is exceeded.

'Student' (1934) has estimated that at least twenty polygenes must control oil content in maize, and many geneticists who have studied continuous variation would tend to put the number higher (see Rasmusson, 1933). Indeed, 'Student' himself was of the opinion that the number of genes in his case was nearer to two hundred than to twenty. This question of the number of genes in a polygenic system is, however, one to which we must return later. It is sufficient for the present to observe that the minimum is several rather than few.

We shall show later (Section 13) that the non-heritable variance of abdominal chaeta number in the cross of B × O was 6·7. The non-heritable standard deviation was therefore about 2·6. Now the lines H and L differed by some 17 chaetae, so that if no more than the minimum of six polygenes were responsible, the average effect of each gene difference would be less than 3 chaetae. The average effect of the individual polygene is therefore at most only of the order of the non-heritable standard deviation. If six is an underestimate of the number of polygenes, their average effect must be correspondingly smaller than the non-heritable component of variation.

The different members of this polygenic system could be distinguished one from another only by their linkage relations. They had similar effects on the phenotype, and the similarity even extended to the gross actions of the whole chromosomes. Further, the effects of the different genes must have been supplementary as well as similar, for no one chromosome, and *a fortiori* no one gene, could of itself account for a difference in number of chaetae so large as that seen between lines H and L. All the chromosomes played their parts, and more or less equal parts, in producing this difference. The same is true of the polygenic system governing heterosis in egg production by *Drosophila* females (Strauss and Gowen, 1943).

The two assumptions that had to be made to link continuous variation to Mendelian genetics have thus been vindicated by experiment. The polygenes responsible are similar in their trans-

mission through the nucleus to those recognized by, and used in, Mendelian analysis. But the differ from these major genes in that they occur in systems, the members of which have small, similar and supplementary effects. Each of these properties has consequences of importance to us. The similarity in transmission makes it possible to use the laws of inheritance established by Mendelian methods for the analysis and interpretation of continuous variation, in the way to be discussed in later chapters. The dissimilarity in effect on the phenotype means that the two types of genes, major genes and polygenes, must play different parts in the control and adjustment of the phenotype. This is particularly true in relation to the action of selection.

Each of the genes normally recognized by Mendelian means appears to be highly specific, in that aside from the special case of polyploids, the part it plays in development can rarely, if ever, be duplicated by other such genes. Or to put it another way, if the gene is not playing its part, the deficiency cannot be made good by other genes of the nucleus. Furthermore, the part played by each gene seems to be so important that when the gene has changed, the mutant form is drastically different from the normal, even to the extent of being completely inviable. Such drastic differences must be disastrous from the standpoint of Darwinian fitness. In short, these genes are of major effect and hence of major importance to the proper functioning of the organism. They seem seldom to have been concerned in specific differentiation (Mat er, 1943*a*), and though their deleterious mutant allelomorphs often occur hidden under the cloak of dominance in wild populations, these mutants are seldom found in homozygous condition outside the laboratory. The homozygotes which will occur by mating of heterozygotes in the wild must be weeded out by natural selection. Such genes are the backbone of the genotype; and being essential like a backbone, the normal allelomorph of each has an unconditional selective advantage over its mutant alternatives.

The polygene is in a different position. It is one of a system whose parts are apparently interchangeable in development. Although individually their effects are not large, the members of a system may act together to produce big differences, as between H and L, or they may act against each other so that similar phenotypes arise from different genotypes, as in O and B. No allelomorph of one polygene has therefore an unconditional advantage over the other; its advantage depends on the allelomorphs present at the other loci of the system. Great potential

variability can lie hidden in the form of the balanced combinations. Equally great genetical diversity and change may occur behind the facade of phenotypic uniformity. Polygenes are the genes of fine adjustment, clothing, as it were, the indispensable skeleton of major genes, and moulding the whole into the fine shape demanded by natural selection. They are the genes of smooth adaptive change and appear to be at the root of speciation (Mather, 1943*a*). They are also the genes with which the plant and animal breeder is generally concerned in his endeavours to produce improved forms.

These special properties of the polygene in selection arise from its relatively small individual effect and from its membership of a polygenic system whose parts are interchangeable in function. Now natural selection acts on the phenotype as a whole, and so the total action of a gene must be small and interchangeable if it is to show the special selective properties of a polygene. A gene which, while contributing to smooth polygenic variation in one character, simultaneously has a further major and more specific effect on another, cannot have the properties of a polygene in respect of its total action, because it cannot be part of a system whose members may balance or reinforce one another in the way on which the special selective properties of a polygene depend. It is therefore of importance to inquire how far the various contributions to polygenic variation in a character are merely the secondary or pleiotropic outcome of gene differences having simultaneous major effects.

Characters which show polygenic variation may also be subject to alteration by major genic effects. The number of abdominal chaetae in *Drosophila* showed only polygenic variation in the experiments which we have discussed, but it is also subject to drastic change by major genes such as 'scute' and 'Hairless'. Indeed, it seems that no character is subject only to polygenic or only to major genic change. All characters may be expected to show both if sufficiently extensive observations are made on them.

In some cases it is possible to see that a major gene is contributing by pleiotropic action to variation which otherwise seems polygenic. Such a case is that of nicotinic acid content in maize grains (Mather and Barton-Wright, 1946). The content of this vitamin varies between strains, and in particular starchy and sugary strains, distinguished by the gene **Su-su**, differ in that the former have on the average only half the content of the latter. Differences among the starchies and among the sugaries are, on

the other hand, relatable to no detectable major genes. It is therefore noteworthy that these apparently polygenic differences are smaller than that produced pleiotropically by the action of **Su-su.** The nicotinic acid contents of the starchy and sugary classes do not overlap even though there is considerable variation within each. Even apart from its simultaneous action on the carbohydrates, the **Su-su** gene would hardly be confused with the polygenic system which is also involved. Its effect is too large.

The results of the *Drosophila* experiment on abdominal chaetae are even more positive. Differences in the second and third chromosomes between the tester stock and any one of the four strains tested, O, B, H and L, could be ascribed to pleiotropic action of the major genes used to mark these chromosomes in the tester. The differences among O, B, H and L in the chromosomes cannot, however, reasonably be ascribed to pleiotropic action of major genes, for no major genes, or at least no known major genes, distinguished them. In other words, the polygenes that were detected cannot be merely major genes exhibiting themselves in a different way.

General observation points the same way. Quantitative differences of the kind which experience has shown typically to be under polygenic control, commonly occur between individuals or strains which are not differentiated by any detectable major gene, even in the most intensively studied plants and animals. Thus whatever transitional stages may exist between them, we must recognize that polygenic systems can and do exist distinct from the major genes.

This conclusion is supported by evidence of another kind. Cytologists recognize two kinds of chromatin within the nucleus, euchromatin and heterochromatin. Special methods may be required to permit the distinction to be observed, and it also seems possible that in some cases the heterochromatin cannot be identified by any known technique. Nevertheless, it is clear that a single chromosome may be partly euchromatic and partly heterochromatic, and that even whole chromosomes may be heterochromatic. The heterochromatin is of special interest because wherever tests have been possible, it has proved to be devoid, or virtually so, of major genes. In this sense it is inert, and it is often so termed by geneticists.

Heterochromatic chromosomes, supernumerary to the normal complement, are known in a number of plants. They must be 'inert' in the above sense, because neither have they an effect

on the phenotype comparable in magnitude with those of extra chromosomes or chromosome parts from the normal complement, nor are they essential to the organism in the way that the normal chromosomes are. Yet their mechanical properties are such that they must either disappear or increase indefinitely in frequency in the absence of some countervailing selective action. That they have not done so is therefore evidence of such action (Darlington and Thomas, 1941), though in what way they affect the phenotype so as to introduce selective differences is generally not clear. Their action is, however, obviously not essential and therefore most probably not unique, and it is equally obviously small in magnitude. They have the properties of polygenes, or more likely of groups of polygenes. The slight effects on vigour and fertility traced in a few cases (Östergren, 1947) accord with this view.

The same is true of the heterochromatic Y chromosome and the homologous heterochromatic portion of the X, in *Drosophila melanogaster*. Apart from the bobbed gene and the male fertility genes, which are confined to the distal half of the Y, both Y and the heterochromatic part of the X are inert in that they contain no genes of major effect. Yet both vary in their effects on the number of sternopleural chaetae, and they vary in a way which points to the conclusion that the variation is due to a number of genes having small, similar and supplementary action; in fact, to polygenes (Mather, 1944). Thus although heterochromatin contains few or no major genes, it is polygenically active. The cytological and genetical evidence agrees therefore in showing that polygenes must be capable of existing as a class distinct from major genes.

Heterochromatin has another property which now becomes of interest to us. It does not always divide cleanly at mitosis. There is reason to believe that, at least under extreme conditions, one daughter nucleus may receive more than the other (Darlington and La Cour, 1941). Polygenic systems carried by heterochromatin may therefore show a certain measure of disorderly behaviour. This would presumably appear as mutation in genetical experiments, but mutation which affected the linear arrangement. Later, crossing-over in an individual heterozygous for such a structural mutation would lead to further variation by producing duplications and deficiencies.

Now duplication or deficiency for a small number of polygenes is not likely to be unconditionally deleterious. The small effects of the change could be balanced by other members of the polygenic system. By this means the heterochromatin might well assume a

somewhat disorderly genic structure. Allelomorphs might not be of like action in development, in the way that is clearly obligatory for the major genes of the euchromatin. Crossing-over of such allelomorphic, but genetically non-corresponding, heterochromatin would bring together, side by side in one chromosome, unlike genes which were not so together in the parent chromosomes. Since we know from the observation of position effect, that adjacent genes may affect one another's action, we may thus have possibilities of variation in polygenic systems borne by the heterochromatin wider than those which have been established from the study of major genes.

Although major genes are confined to euchromatin, polygenes are not confined to heterochromatin. The X chromosome of *Drosophila melanogaster* is divided into a proximal heterochromatic and distal euchromatic portions. A survey of its polygenic activity shows no corresponding division (Wigan, 1947). Polygenic variation is associated more with the ends of the chromosome than it is with the middle. It occurs in euchromatin as well as heterochromatin, and indeed is greater in the euchromatic left end than in the heterochromatic right.

It does not seem likely that this activity of the euchromatin is to be accounted for by the inclusion of small pieces of heterochromatin within it. Such pieces are known to be present in the otherwise euchromatic end of the X chromosome, but the distribution of polygenic activity does not seem to be associated with their positions. Rather it appears that polygenes, as well as existing separately from major genes, may exist side by side with them. Indeed, it is difficult to see how the euchromatin at the left end of the X chromosome could show such polygenic variation as it does without the association between polygene and major gene being in this case a close one. It would appear likely that at a locus recognized as that of a major gene by the existence of a major discontinuity of effect between two allelomorphs, there can also exist allelomorphs differing in action only in the way typical of polygenes. If this is so the major gene must be so constructed that it may vary to produce on different occasions a major and specific change of action, and a smaller non-specific change.

A gene that can vary in these different ways, must have a corresponding complexity of parts. Furthermore, the assumption is difficult to avoid that the drastic and unique effect of the change, which leads us to class the gene as major, must imply a

greater disarray of parts or a disarray of more parts than does the smaller effect, reproducible by other genes, by which we recognize the polygene. On this view the polygene represents a simpler difference of the structure than does the major gene, a dissimilarity in one or a few of the parts, the rest working normally, as opposed to dissimilarity of many parts, or, perhaps more likely, of the integration of these parts. Again, since the simpler dissimilarity may apparently be shown at a number of loci, while the more complex one is unique, one is led to suppose that the structures at different loci often contain the same parts. If this is the case the genes must then owe much of their individuality to their organization, just as two proteins might contain similar amino-acids yet differ in properties because these amino-acids were carried in different proportions and in different arrangements (Mather, 1946*b*).

The relations possible between polygenes and major genes affecting variation in the same character are therefore two. While affecting the same character they can be distinct classes of gene, the one having nothing to do with the other. In other cases, however, it would appear likely that different variations of the same structure can lead to its being recognized both as a major gene and as a polygene, though on different occasions. This second relation is nevertheless not one of pleiotropy in action, because the polygenic effect is observed when the characteristic major genic effect is absent. Rather it follows from a complexity of structure and mutation. Thus in either case, whether the difference which we recognize as part of a polygenic system is due to change in a structure which can never be associated with a major genic difference, or whether the difference recognized as due to a polygene is consequent on change in a structure which can also change independently so as to appear as a major gene, the small nonspecific difference is not a mere secondary action of any major difference. Though in any given instance the polygene may or may not exist as a physical structure independent of major genes, its difference can always exist as the determiner of variation independent of any major discontinuity. It will then contribute only to the polygenic variation and will be acted on by selection only as part of a polygenic system.

CHAPTER 2

CHARACTERS

4. PHENOTYPE AND GENOTYPE

IN GENETICS, the term character is applied to any property of an organism in regard to which similarities or differences, especially those of a heritable nature, are recordable between individuals. A great variety of characters is now known to show heritable variation. It includes gene and chromosome behaviour, cell shape, gross morphology, physiological and biochemical properties, psychological characteristics, mating behaviour, resistance to disease and toxic agents, ability to infect a host, ability to act as a vector in virus transmission, and antigen production. Indeed, few geneticists would question the proposition that no character of an organism would fail to show heritable variation, were it subjected to adequate examination. Furthermore, the magnitude of the heritable differences shown in a character may range from the smallest that is detectable to the largest that is possible.

Our discussion of the genetical basis of continuous variation has already led us to touch on the relations between gene and character. Three principles have emerged, whose consequences we must now examine in more detail.

The first of these, and one which we owe to Johannsen, is the principle that the phenotype is the joint product of genotype and environment. Variation in a character may therefore result from variation in either genotype or environment, and, as Johannsen showed, the two kinds of variation in the character, heritable and non-heritable, cannot be distinguished by mere inspection. A plant or animal may be small because of insufficient feeding or because of its ancestry, and only a breeding test can distinguish the one situation from the other.

The second principle is that the variation in a character may be caused by alteration in any of a number of genes. The genes causing differences in the same character may be related in various ways. They may, of course, all be members of a polygenic system, as in the case of the genes affecting the number of abdominal chaetae in the four strains of *Drosophila*. They may, on the other hand, all be major genes whose effects on the character are neither small nor supplementary. The changes produced by such major

genes may be distinguishable by inspection, as is the case with many genes affecting for example flower colour in plants or eye colour in *Drosophila*; or the changes may appear alike. Many chlorophyll-deficient barley plants, for example, look alike though caused by different genes, and the same is true of 'minute' bristles which can be brought about by any of some 70 known genes in *Drosophila*. Again, variation may at the same time be due partly to a major gene and partly to a polygenic system as we saw with the nicotinic acid content of maize grains. Thus, not only is a breeding test necessary to show how far the difference between two individuals or strains is heritable, it is also necessary to reveal the nature and relations of the genes determining any heritable differences that may be found.

Furthermore, just as two individuals or strains may owe their difference to any of a number of genes, two which are alike may owe their similar phenotypes to different genes. This applies to comparisons between species as well as within them. In cotton, for example, *Gossypium hirsutum* and *G. barbadense* each have forms with large coloured spots on the petals. They look alike, but breeding tests have shown that the genetical architecture of the character is different in the two cases (Harland, 1936). Indeed, Harland regards such differences of genetical architecture as both widespread and of great importance in evolution.

The genetical basis of any one of a range of phenotypes can be discovered only by breeding. Sometimes a single test will serve to clarify the situation once and for all, because where the responsibility of a major gene is demonstrated, the genotype can be inferred subsequently from the phenotypic appearance within the range for which the difference has been established. This is, however, never possible where the variation is under the control of a polygenic system, because of the regular interchangeability in effect of the polygenes both with one another and with nonheritable agencies.

The third of our principles relating to gene and character is that just as one character may be influenced by more than one gene, one gene may influence more than one character. The simultaneous effect of the **Su-su** gene on carbohydrates and nicotinic acid in maize has already given us one example of this property of pleiotropic action, which is indeed quite common among genes. Sometimes the connexion between the various effects is fairly obvious. Genes initially detected in plants by their effects on leaf shape are often, for example, observed also to affect the shape of

the petals. This is to be expected, but other associations are more surprising. A series of four allelomorphs affect the size of the coloured 'eye' round the mouth of the corolla tube in *Primula sinensis*. So far as is known this is the only effect of three of them, but the fourth also shortens the style in such a way that flowers, otherwise of the pin type, become homostyled. This is all the more remarkable because the effect of this fourth allelomorph on the eye is indistinguishable from that of one of the other three, which itself causes no shortening of the style.

The common developmental origin of a number of apparently unrelated changes caused by one gene is sometimes made clear by embryological studies. A recessive gene is known in the rat which kills the animals soon after birth. The affected animals show a wide range of peculiarities. These are especially marked in the circulatory and respiratory systems, but also appear in the form of the snout, the occlusion of incisor teeth, and the ability to suckle. When traced back, however, they all arise from an initial breakdown of the cartilage (Grüneberg, 1938). The complexity of the gene's pleiotropic effects is thus traceable to a single initial action, which changes the general course of development and so leads to the gene change expressing itself in a syndrome of varied abnormalities.

The longer the chain of events between the first action of the gene and its final expression in the phenotype, the greater the complexity of effects which can arise from a simple alteration. Conversely where a character, such as the production of a particular antigen, is a more immediate expression of gene action, a simpler correspondence would be expected, as is indeed observed, between gene change and character change. Much, though we cannot say all, of the complexity of relations between gene and character is thus to be attributed to the multiplicity of stages intervening between initial action and final expression. It is a multiplicity which permits one gene to show a number of apparently different effects and at the same time offers opportunity for its consequences, and with them the character, to be changed by other genes and by external agencies. This would be so even if as Beadle (1945) suggests, each gene has a single primary action and each primary action is characteristic of one gene. The complexity will be all the greater if a given gene can in fact have more than one primary action and if, as is suggested by polygenic systems, the same primary action can be shared by a number of genes.

It is therefore clear that while a given phenotype, taken as

a whole, can be related to a given genotype, acting as a whole, in a given set of circumstances, no similar correspondence can be expected between the parts of the phenotype and the parts of the genotype: the genes of a nucleus must be related in action, and the characters must be related in development.

This complexity of relation between genes and characters has many consequences outside the scope of our present discussion. It has, however, an immediate importance for us because, as we shall now see, it determines the lines to be followed in making the genetical analysis with which we are concerned.

5. GENETIC ANALYSIS AND SOMATIC ANALYSIS

The object of most genetical experiments has been to throw light on the organization and transmission of the genotype. In such experiments the relations between the genes which are used and their phenotypic expressions are of secondary interest. They are of importance only in so far as they limit recognition of the genotypes, with which the experiment is primarily concerned, through the changes they produce in the phenotype. An unfortunate choice of 'marker' genes, whose effects are indistinguishable or overriding, results in confusion of the phenotypes associated with certain of the genotypes. Genotypic classification is thereby made less complete and information is lost. The most useful choice of genes is clearly that which, in an appropriately arranged experiment, leads to each of the possible genotypes giving rise to a unique and distinctive phenotype. All the genotypes can then be identified with confidence and analysis is complete. In this type of experiment, therefore, the nature of the changes brought about by the genes in the phenotype is of purely technical interest. Emphasis is on the analysis of the genotype rather than the adjustment of the character.

In the practical utilization of genetical knowledge, however, the main interest centres in the adjustment of the character. The plant breeder is basically concerned with improving yield, disease resistance, or some other feature of his crop, and he seeks knowledge of the genetical architecture of the character with that end in view. He cannot choose the genes with which he will work: he is concerned with all the genes that contribute to the variation in the character, whether they are major genes or polygenes and whether they have pleiotropic effects or not. He therefore requires analytical methods capable of dealing with all types of genetical variation.

In so far as there are discontinuities in the variation of the character, the breeder can use the methods of Mendelian genetics. The inheritance and interactions of any major genes recognizable in this way can be ascertained, and by appropriate breeding any desired type can be produced and recognized at will. The success of this approach derives from the recognition and isolation of the genes involved, so that they may be put together in any way that is desired. It may be found that a given phenotypic difference can be produced by more than one gene; but, as Nilsson-Ehle has shown us with his cereals, we can still isolate the genes one from another and put them together in the way we wish, provided they can be recognized as units through their production of discontinuities in the variation of the character.

The application of the Mendelian type of analysis can be extended by special methods, notably the use of inbred lines, to permit the examination of particular gene differences on a uniform, or isogenic background. In this way the obscuring effects of other gene segregations may be removed and genes recognized by the production of discontinuities in variation which would otherwise have been swamped by the mass of segregation. This method is powerful, but it is also laborious and expensive of time. Furthermore, even were we prepared to adopt these measures for the analysis of any polygenic systems governing variation in the character in which we are interested, we should still come up against two further limitations. In the first place, though it might be possible to analyse the system using a series of individually isogenic lines, the synthesis of any desired type by crossing these lines would bring back all the difficulties which arise from the obscuring effects of the genes on one another's segregation. The second and perhaps more important limitation is imposed by the obscuring effect of non-heritable variation on the segregation of polygenes. This is not, of course, eliminated by the use of inbred lines. In fact the inbreeding depression seen in organisms which normally cross-breed may be accompanied by an increase in non-heritable variation.

While, therefore, we may conceive of all genes as capable of isolation and handling by Mendelian means, given an ideal uniformity of genetical background and elimination of non-heritable variation, we must in practice accept a situation falling short of this ideal. We can reduce non-heritable variation but at present we cannot eliminate it. Indeed, we have no certainty that it can ever be wholly eliminated even under the most rigorously

controlled conditions. Equally, the most inbred stock still has a residuum of genetical variation, if only from mutation. Finally, even could we achieve this ideal state in which genes of small and like effects could be separated and handled by the Mendelian method, the cost in both labour and facilities would prohibit its general use in handling polygenic variation for practical purposes. We must thus be prepared to accept the situation in which genes, though contributing as units to the variation, are not recognizable as such by means of their effects on the phenotype and therefore are not separable in analysis. The genetical analysis is thus limited by the complexity of relation between gene and character.

Attempts have been made to simplify the genetical analysis by a prior somatic analysis of the character. Many characters, such as yield in crop plants, can be regarded as made up of a number of sub-characters. If each of these were under separate genetical control, the inheritance of its variation would be simpler than that of the whole character. A preliminary somatic analysis would then make the genetical analysis easier.

Consideration of the relations between genes and characters has shown us, however, that there is no ground for expecting the sub-characters that we can recognize in the phenotype to be under completely separate genetical control. Rather we must expect that some genes will affect both of any pair of sub-characters while others may affect only one of the pair. The variation of the two will then be correlated but not completely so, and the degree of correlation cannot be predicted. It will depend on the sub-characters in question and on the genes which are contributing to the variation.

This basis of the partial correlation can be illustrated from the action of major genes on flower colour (see Beadle, 1945). This depends on the presence of anthocyanins and anthoxanthins, among other pigments. Now genes are known which affect the type and intensity of anthocyanin pigmentation while having relatively little effect on the anthoxanthin. Others in turn change the anthoxanthin pigmentation while leaving the anthocyanins almost unaffected. It appears, however, that the two kinds of pigment have a common precurser. Thus, even in addition to any negative correlation between variation in the two types of pigmentation which may arise from competition for this precursor, a positive correlation may be produced by change in the action of genes whose chief effect is on the supply of precursor. The degree of correlation observable in any group of individuals will depend therefore on the developmental relations between the two types

of pigmentation and on the genes which happen to be contributing to the variation. Furthermore, where the variation is due to immediate segregation, the linkage relations of the genes must also have its effect on the correlation.

In the case of variation determined by major genes, the correlation between the sub-characters, in this case anthocyanic and anthoxanthic pigmentation, need cause us no concern. We can isolate the genes, determine the individual effects of each and also discover their interactions in effect. The partition of the character into its constituents even helps us with the genetic analysis to the extent that it enables us to distinguish the various genes through the differential effects on the sub-characters which we can establish by experiment as characteristic of each. The analysis is, however, primarily into units of inheritance. The somatic analysis is built on to the genetic analysis and must be justified at every stage by reference to it. Pleiotropic action and linkage can then be detected, and the subsequent use of somatic analysis can be confined to effecting the distinction between those genes which it has shown itself in practice as capable of distinguishing.

The situation is quite different when the genes themselves cannot be isolated in experiment. Somatic analysis cannot then be founded on a prior genetic analysis and its limitations therefore cannot be gauged. Though the sub-characters may each have a simpler genetical basis than the whole character, we know neither how many genes have been separated by the analysis into sub-characters nor how far any failure to achieve separation depends on linkage as opposed to pleiotropic action. Furthermore, each sub-character will still be showing the effects in its variation of an unknown number of individually unrecognizable genes. Such a somatic analysis does not simplify the genetical problem. When it has been made we still have to deal with a system of genes affecting each sub-character and we do not know how far these systems are interlocked.

It may be argued that genes which affect the same character but do so by altering different sub-characters, cannot be regarded as members of the same polygenic system, because the similarity of their effects is only superficial. However this may be theoretically, we have no practical means of distinguishing them from genes which affect both sub-characters simultaneously. Thus where we are dealing with continuous variation, due to genes which we cannot expect to be readily recognizable as individuals in segregation, we do not overcome the intrinsic difficulty of the situation

by attempting a prior somatic analysis. We must in any case use methods capable of dealing with systems of genes taken as wholes, and we may as well do so first as last.

Somatic analysis is therefore of use for genetical purposes only where experiment has shown its application to be both justifiable and helpful. So far we have been considering it as a possible help for genetical analysis and we have seen that this justification can be given for its use in sorting out major genes, but that it cannot be expected to be of great help in the sorting out of genes governing continuous variation. It may nevertheless have its uses in predicting the average breeding behaviour in respect of a character showing such continuous variation.

Yield in wheat plants may be regarded as built up from the sub-characters, number of ears, number of grains per ear and average weight per grain. The genetical variation of these characters has been shown by Smith (1936) to be correlated in Australian varieties. The somatic analysis does not therefore simplify the methods which must be used in genetical analysis of the polygenic system affecting variation in yield. It does, however, aid us in partially disentangling the heritable and non-heritable variation. Plants may have the same yield but have it for different reasons. Excellence in one sub-character may be balanced by poor performance in respect of another. In so far as these balancing effects are genetic the somatic analysis does not of itself help us to predict breeding behaviour in respect of yield as such (though, of course, it may help in so far as we are concerned to breed for, say, ear number as a desirable character in its own right, rather than as a mere sub-character of yield, in which capacity its improvement is no more a desirable means of increasing yield than is the improvement of any other sub-character). But where the genetical quality is being obscured by non-heritable effects somatic analysis may help us, because one or more of the sub-characters may show relatively less of this obscuring variation than the character does as a whole. In his wheat, Smith found that this was indeed the case. In particular knowledge of the sub-character, average weight of the grain, was helpful in lessening the error caused in the assessment of the genotype from the phenotype by the intervention of non-heritable agencies. Prediction of breeding behaviour in respect of yield was therefore capable of improvement by use of the somatic analysis into sub-characters, information about any of which could be used to supplement that about yield as a whole.

The value of somatic analysis for the improvement of prediction

of average breeding behaviour cannot of course be assumed without evidence, any more than its value in aiding genetical analysis could be assumed without evidence. It requires experimental justification each time. Smith supplied this by his observation that average weight of grain showed relatively less non-heritable variation than did the yield as a whole. Somatic analysis may prove to have other genetical uses, but in every case its application must be based on adequate genetical evidence.

6. THE COMBINATION OF CHARACTERS
(in collaboration with Dr. U. Philip)

Just as a character may be capable of resolution into a number of sub-characters, it may itself be only one of a number of characters in whose joint properties we are interested. Thus yield is only one of a number of features on which the general merit of a wheat plant depends. Baking quality, straw properties and resistance to various diseases are other characters which the breeder cannot ignore. These, together with yield, may be taken as the components of a super-character which is itself the overall merit of the plant.

Just as two sub-characters may not be independent in their genetic control, whether for physiological or mechanical reasons, so we may not be in a position to treat any two characters themselves as independent. And just as the division into sub-characters does not simplify the genetical problem posed by continuous variation in such a way as to permit the use of simpler genetical methods, so the aggregation of characters into a super-character does not necessitate the use of more complex genetical methods in its analysis. The two relations, of sub-character to character and of character to super-character, differ in one respect however. The former is a precise relation since the character is itself definable and measurable, while the latter is not precise because the super-character is not generally capable of final definition and measurement. The method of combining the characters is not clear. Thus the yield in grammes per wheat plant, for example, is obviously the product of the number of ears, the average number of grains per ear and the average weight in grammes per grain. But what coefficients should be given to yield, baking quality, &c., in arriving at a measure of the super-character, overall merit? And by what method should they be combined?

If some measure of overall merit were available the problem could be solved by treating this as a single character, with yield, &c., as sub-characters. Then for any range of material the multiple

regression of overall merit on its sub-characters could be found and used as a means of predicting or estimating merit from the measurements of its components. The question is therefore that of finding a measure of overall merit. It would be possible in this case to use the free market price of the produce from a variety as such a measure. The objections to such a course, fluctuations in price even when taken relative to some standard, and the undesirability of confining attention to the features which preponderantly determine market price at any given moment, are too obvious to require stressing, even where free markets exist. Nevertheless this means of evaluating the emphasis placed by commerce on the various qualities of a biological product may well have its uses, albeit limited uses.

Another and more generally applicable means of combining characters into a single measure is afforded by Fisher's discriminant functions. These bear a formal resemblance to multiple regression equations, although their aim is a different one. They may be illustrated by reference to Smith's consideration of yield in wheat, mentioned above. The phenotypic relations of the various sub-characters of yield to yield itself are clear and fixed. Since, however, yield and its sub-characters are subject to non-heritable variation, the relations of genotypic value, in respect of yield, to the various phenotypic measurements are not so obvious. The problem is that of how best to predict the genotypic value in respect of yield, as opposed to yield itself, from the phenotypic observations on the sub-characters. This Smith has approached by finding a function of the phenotypical measurements, number of ears, number of grains per ear and mean weight per grain, selection for which would give the maximum advance in yield. In this way coefficients are calculated for the combination of the sub-characters with one another in the way most useful for the purpose in hand. The function obtained, as the best for discriminating between genotypes, is a discriminant function.

Discriminant functions are linear functions of the various measurements which are available and which may themselves be correlated with one another. The coefficients given to the various measurements in the function are chosen so as to maximize the differences between two or more classes of objects or individuals relative to the variation within the classes. In other words, they afford the best available means of discriminating between the classes. The theory of these functions has been described by Fisher (1936, 1938) and they have been used for a variety of purposes as

different as the classification of Egyptian skulls, the selection of wheat plants, the recognition of different species and the investigation of the psychological qualities essential in a good salesman. The estimation of the coefficients by which the measured quantities are combined is at its simplest where the discrimination is between only two classes; but the calculations necessary in a more complex case, as well as the way in which the functions can be used, may be illustrated by reference to a genetical experiment on ear conformation in barley. We are indebted to Professor R. A. Fisher for his help with the calculation of this discriminant function.

The object of the experiment was the investigation of the genetical control of ear conformation in barley. The conformation of the ear depends on its absolute length, its absolute width and the absolute lengths of the internodes between the spikelets; but it also depends on the relative values which these various measurements bear to one another. It was therefore decided to represent ear conformation by a discriminant function compounded of all three measurements in the way best suited for genetical investigation.

The two varieties Spratt and Goldthorpe were chosen as both having fairly broad ears (Table 4), but as differing in the genetical architecture of their ear characters. No simple Mendelian differences could be detected between the varieties: variation was continuous in the F_2 raised from the cross Spratt × Goldthorpe. Two ears were taken from each of 170 plants of this generation. On each ear three measurements were made, all in millimetres, viz. the overall length, neglecting the awns (L), the maximum breadth (B) and the combined length of the central six internodes (C). The averages of these three measurements are given for the parent and F_2 in Table 4. The analysis of variance of L, and the results of the

TABLE 4

Mean Values of L, B, C, in millimetres and X in the Barley Parents, F_1 and F_2

	L	B	C	X
Spratt	70·2	10·2	28·6	321·7
Goldthorpe	70·5	11·3	27·8	302·3
F_1	85·3	10·1	33·6	398·0
F_2	78·1	10·3	31·1	358·2

$X = L - 9B + 12C$

analyses of variances and covariances of all three measurements on the 340 F_2 ears, into the two parts, between and within F_2 plants respectively, are given in Table 5.

TABLE 5

Analysis of Variance of L

	S.S.	N.	M.S.	V.R.
Between plants	91385·438	169	540·742	8·597
Within plants	10692·500	170	62·897	
Total	102077·938	339		

Mean Squares and Mean Cross Products between and within 170 F_2 Barley Plants

	N	L^2	B^2	C^2
Between plants	169	540·742	8·406	72·699
Within plants	170	62·897	1·388	5·038
Variance ratio	—	8·597	6·055	14·429

	LB	LC	BC	X^2
Between plants	−44·503	177·224	−19·658	20990·938
Within plants	0·408	7·612	−0·820	1253·420
Variance ratio	—	—	—	16·747

From these analyses it is clear that L, B and C are correlated in all combinations between plants, and also in all combinations except LB within the plants. The information that each contributes about ear conformation is not independent of the others. Nevertheless, since the correlations are not complete, each can contribute information unique to itself. A measure compounded of all three should therefore be superior in specifying the plants to any one of them taken individually. The problem is, of course, that of finding the most suitable compound.

Leaving out of account the possibility of somatic mutation, the variation within plants is obviously non-heritable. The variation between plants is not wholly heritable, but as will be seen later (Section 18) approximately $\frac{7}{8}$ of it is of this kind in F_2. If therefore a function of L, B and C is found which maximizes variation between plants relative to variation within plants, it should go a long way towards giving a measure of ear conformation whose genetical variation is at a maximum compared with at least one important kind of non-heritable variation.

The coefficients b_L, b_B and b_C of L, B and C respectively in this function are found from the equations

$$b_L(A_{LL}-\phi a_{LL})+b_B(A_{LB}-\phi a_{LB})+b_C(A_{LC}-\phi a_{LC})=0$$
$$b_L(A_{LB}-\phi a_{LB})+b_B(A_{BB}-\phi a_{BB})+b_C(A_{BC}-\phi a_{BC})=0$$
$$b_L(A_{LC}-\phi a_{LC})+b_B(A_{BC}-\phi a_{BC})+b_C(A_{CC}-\phi a_{CC})=0$$

where A_{LL}, A_{BL}, &c., are the total sums of squares of L, total sums of cross products of L and B, &c., a_{LL}, a_{LB}, &c., the corresponding sums of squares and cross products between plants and ϕ is an adjustable quantity whose value is to be estimated. The necessity for estimating ϕ leaves, of course, only two equations relating to the three b coefficients. We can therefore estimate only their relative magnitudes.

ϕ is first estimated from the condition, implicit in the equation, that the determinant

$$\begin{vmatrix} A_{LL}-\phi a_{LL} & A_{LB}-\phi a_{LB} & A_{LC}-\phi a_{LC} \\ A_{LB}-\phi a_{LB} & A_{BB}-\phi a_{BB} & A_{BC}-\phi a_{BC} \\ A_{LC}-\phi a_{LC} & A_{BC}-\phi a_{BC} & A_{CC}-\phi a_{CC} \end{vmatrix} =0$$

substituting for A_{LL}, a_{LL}, &c., this becomes

$$\begin{vmatrix} 102077{\cdot}9-91385{\cdot}4\phi & -7481{\cdot}6+7521{\cdot}1\phi & 31244{\cdot}9-29950{\cdot}9\phi \\ -7481{\cdot}6+7521{\cdot}1\phi & 1656{\cdot}6+1420{\cdot}6\phi & -3461{\cdot}8+3322{\cdot}3\phi \\ 31244{\cdot}9-29950{\cdot}9\phi & -3461{\cdot}8+3322{\cdot}3\phi & 13142{\cdot}6-12286{\cdot}1\phi \end{vmatrix} =0$$

which gives the equation in ϕ

$$264721-607468\phi+458060\phi^2-113720\phi^3=0$$

The derivation of this equation can be found from any text-book of matrix algebra.

The lowest root, $\phi=1{\cdot}06196$, is the solution needed. Substitution for ϕ in the original equation then gives

$$5030{\cdot}26b_L+505{\cdot}51b_B-561{\cdot}76b_C=0$$
$$505{\cdot}51b_L+147{\cdot}98b_B+66{\cdot}35b_C=0$$
$$-561{\cdot}76b_L+66{\cdot}35b_B+95{\cdot}25b_C=0.$$

These are satisfied by $\frac{b_B}{b_L}=-8{\cdot}812$ and $\frac{b_C}{b_L}=12{\cdot}036$.

Then, putting $b_L=1$, the desired discriminant function representing ear conformation becomes

$$X=b_LL+b_BB+b_CC$$
$$=L-8{\cdot}812B+12{\cdot}036C$$

which is approximated sufficiently well by the more easily applied function

$$X = L - 9B + 12C$$

Then $$X^2 = L^2 + 81B^2 + 144C^2 - 18LB + 24LC - 216BC$$

from which its analysis of variance may be written down as shown in Table 5. Whereas L^2, B^2 and C^2 gave variance ratios of 8·597, 6·055 and 14·429 respectively, X^2 gives a variance ratio of 16·747. The variation between plants relative to that within plants has been raised by some 16% over the previous best. It is worth noting that C, which gives the previous best variance ratio, has the highest coefficient in the formula of X.

Ear conformation is not a character which can be measured directly. In fact, before we could measure it we had to define it. This was done by specifying that it should be compounded of ear length, ear width and ear internode length in such a way that the compound would maximize the variation between plants, which is largely genetic, relative to that within plants, which must be almost wholly non-genetic. We thus combined the three characters into a single super-character, defined in a way specially suited to our needs.

The method is open to use whenever it is possible to represent our needs in the form of a maximization of differences between recognizable classes, in our case the F_2 plants. It may therefore be applied to the definition of characters which are either undefinable or not capable of measurement by other means. Thus flavour in fruits is generally incapable of measurement. We can, however, measure such characters as pH, and sugar concentration. If we can classify a range of fruits into good and poor, it is possible to calculate the discriminant function of pH, sugar concentration and such other measurements as are available, which maximizes the difference between the flavour classes. In this way a usable definition can be given to the flavour character. The definition cannot be a final one, since some new measurable property of the fruit may be observed which supplements or even partially replaces one or more properties of which an earlier discriminant has been compounded. The success of a discriminant function in achieving the desired discrimination can be tested in a simple way (Mather, 1943*b*) and so compared with that of its predecessors or successors.

Where different classifications have led to the calculation of different discriminants by unlike combinations of the same ingredients, a further investigation is possible. We may consider

a discriminant as a vector specifying the deviation of one class from another in a space of as many dimensions as there are measured properties used in making the discriminants. Discriminants may therefore be regarded as differing in either direction or distance or in both. Methods are available for separating these two kinds of difference (Fisher, 1938). These have yet to be used in practice, and indeed the statistical development of discriminant functions has in general outrun their practical application. These functions promise to be a powerful tool for use in combining and analysing metrical characters in genetical work and to offer means of overcoming many of the difficulties met with in the past.

CHAPTER 3

SCALES

7. THE PRINCIPLES OF SCALING

WITH DISCONTINUOUS VARIATION such as that produced by genes of major effect, individuals are assignable to one or other of a number of distinct classes. The resulting data consist of the frequencies with which the various classes are represented in the group of individuals under examination. The characteristics by which these alternative classes are recognized may also be expressed, if we so wish, in terms of some convenient metric or metrics, but this is not essential for the analysis of the data. To take an example, the tall peas of a segregating family such as Mendel considered may average 6 feet in height and their short companions may be only 2 feet high; but it would make no difference to the treatment of the frequency data if the talls were 10 feet as against only 1 foot for the shorts. Nor would the analysis be different if the talls were only 4 feet with the shorts up to 3 feet provided that each individual fell unambiguously into one class or another. The analysis requires no assumptions about the metrical relations of the classes, because the genetical situation in question is completely described by the frequencies of talls and shorts. The only essential is that the shortest tall be clearly taller than the tallest short; that, in fact, the distribution of heights be discontinuous. Granted that this is so, the precise metrical relation between the characters of the two classes is a matter of indifference so far as the treatment of the frequency data goes. The result would in fact still be the same if the plant heights were expressed in the terms of log feet, or antilog feet, or indeed on any reasonable scale, for the discontinuity would still exist and the regular separation of the classes would still be possible. With frequency data therefore there is no problem of choice of scale.

With continuous variation the situation is different. Each observation is a measurement which must be regarded as potentially unique, even where, owing to the shortcomings of our measuring instruments, two or more individuals appear in the records as having the same measurement. Without regular discontinuities we have no natural means of grouping into frequencies, and each datum can have no significance other than as measurement of

character expression. Thus the usual variation of human stature cannot be described adequately for genetical purposes by saying that there exist so many talls and so many shorts. Such a classification has no genetical foundation. Even if the variation were to be expressed in the form of frequencies of individuals with heights lying within successive ranges of, say, 1 inch, the representation would still be inadequate because the classification into these ranges is justified by neither genetical nor biometrical considerations. Indeed, some of the biometrical characteristics of the variation are distorted by such artificial grouping.

We are therefore in the position of necessarily using biometrical quantities, means, variances, &c. to replace frequencies in describing the variation. Now the resulting description can be valid only in terms of the scale on which the measurements are taken. A change of scale by, say, the logarithmic transformation, will change the values of these descriptive quantities, and it will change them unequally for measurements of different magnitudes. The description of such a body of data, and *a fortiori* the comparison of two or more such bodies of data, must therefore depend on the scale used in measurement. Clearly the choice of an appropriate scale is the first step in the analysis of polygenic variation.

The scales of the instruments which we employ in measuring our plants and animals are those which experience has shown to be convenient to us. We have no reason whatsoever to suppose that they are specially appropriate to the representation of the characters of living organisms for the purposes of genetical analysis. Nor have we any reason to believe that a single scale can reflect equally the idiosyncrasies of all the genes affecting a given character. We cannot even assume without evidence that a scale appropriate to the representation of variation of a character in one set of individuals under one set of conditions will be equally appropriate to the representation of that same character either in a different set of individuals, which may be heterogenic for different genes, or under different conditions. It may, therefore, never be possible to construct an *a priori* scale for the representation of variation in a character. Certainly with only our present knowledge of gene action, the construction of such a scale is impossible. Observations already available, such as those of Powers (1941) serve merely to emphasize this conclusion.

The scale on which the measurements are expressed for the purposes of genetical analysis must therefore be arrived at by empirical means. It should obviously be one which facilitates both

the analysis of the data and the interpretation and use of the resulting statistics. Now sums, sums of squares, and sums of cross-products, on the partitioning of which the analysis must depend, may be most conveniently regarded as composed of various components, individually determined by the various genes and by the non-heritable agents, which are summed to give the quantity in question. The scale should therefore be one on which such summation of effects is possible. Ideally this would mean that any given gene substitution would lead to the same phenotypic difference no matter where on the chosen scale its effect was measured. The replacement of **A** by **a** or of **B** by **b** in any genotype and under any set of environmental conditions should make the same difference, no matter what the measurement associated with the original genotype and conditions might be. In the same way the difference associated with the action of any non-heritable agency should be independent of the effects of other agents, heritable and non-heritable. The genes and non-heritable agents should all be additive in action.

In practice, of course, such a scale may be impossible to find. Each gene and each non-heritable agent may be acting on its own unique scale, or at least all may not be acting on the same scale. Since, however, the genetical analysis cannot pretend to determine the individual effects and properties of genes which are not individually distinguishable, it should suffice to discover a scale on which the genic and non-heritable effects are additive on the average, so far as the data go. But having found such a scale, its limitations must not be forgotten. The discovery of a scale which is suited to our needs empirically cannot of itself justify theoretical conclusions concerning the physiology of gene action, though it is, of course, legitimate to test the agreement of any empirical scale with one expected theoretically from other considerations of gene action.

8. THE CRITERIA OF SCALING

A scale empirically adequate for analytical purposes must satisfy two criteria. On it the genic effects must on the average be simply additive; and the contribution made by non-heritable agents must be independent of the genotype. If the chosen scale is deficient in either or both of these ways, the statistics by which the distributions are described will be distorted in greater or less degree.

Allowance may be made for the lack of additiveness due to

genic interaction by the inclusion of a special term in the analysis; but the wide range of possible interactions makes the interpretation of this term and its use in predicting further breeding-behaviour far from clear. Non-additiveness of effect may of course also be shown by allelomorphs of the same gene. Such dominance, however, covers a more limited range of relations than does interaction and so it presents little difficulty in either analysis or interpretation and prediction. Furthermore, we have no reason to expect that a scale which eliminates or minimizes the effects of interaction, will also eliminate or minimize the effects of dominance. We must therefore aim at removing, so far as is possible, the effects of interaction between genes, allowing dominance to take its own value on the scale so reached. The success of this policy will be discussed in Section 24, when we come to consider in more detail the phenomena of dominance and interaction as they appear in biometrical genetics.

As we have seen, it is always possible that no scale can be found which wholly removes non-additiveness in effect of various contributions made to the variation by either the genic or the non-heritable agencies; but the adoption of a scale which at least minimizes and balances such bias, by satisfying so far as is possible the criteria of additive effects, must always make interpretation easier and more confident. A small departure from additiveness is not in any case likely to engender serious difficulties or errors. It may well be that the computational work involved in re-scaling would be uneconomical under these circumstances, particularly if the deficiencies of the scale in use were not discovered until late in the analysis. Such a case is afforded by the investigation of ear conformation in barley, discussed in Sections 18 and 21.

The tests of satisfaction of the two criteria by a scale can never be final. They can never do more than detect departures from additiveness greater than a certain minimum—a minimum which must itself vary with the range of variation in the character covered in the tests, and with the number of observations available. A scale satisfactory over any given range of variation must generally be satisfactory over any narrower range contained therein and for any smaller body of observations. It cannot, however, be assumed to be satisfactory over a still wider range of variation or for a larger body of data.

These limitations apply more particularly to the use of a scale derived from one set of observations for the analysis of another set, without further testing of the scale. Within a body of results the

scaling tests will be made over the range that will be involved in analysis and their limitations will not therefore become sources of error. In a word, the potential danger lies not in using an empirical scale for the analysis of the data from which it was derived, but in the general application of an empirical scale to data which have not been tested for conformity. In using a scale which is empirically obtained, due tribute must be paid to the limitations of the empirical method.

The additiveness of genic effects required by the first criterion of scaling may be tested by comparisons of the mean measurements of backcross, F_2 and F_3 families with each other or, better still with those of the true breeding parental lines and the F_1 from which the segregating generations have been derived. In respect of any one gene, the backcrosses (B_1 and B_2) of F_1 to the true breeding parents (P_1 and P_2 respectively) will consist of individuals half of which are heterozygous like the F_1 and half homozygous like the parent. Where the effects of a number of such genes are additive on the average, the mean measurement of B_1 must fall halfway between the mean measurements of F_1 and P_1, provided that no complications are introduced by differential viability or fertility. This relation may be expressed as

$$\bar{B}_1=\tfrac{1}{2}(\bar{P}_1+\bar{F}_1)$$

and similarly
$$\bar{B}_2=\tfrac{1}{2}(\bar{P}_2+\bar{F}_1)$$

where $\bar{B}_1$ is the mean measurement of the individuals in the backcross B_1, $\bar{P}_1$ the mean of P_1, $\bar{F}_1$ that of F_1, and so on.

On the same assumptions it is not difficult to see that

$$\bar{F}_2=\tfrac{1}{4}(\bar{P}_1+\bar{P}_2+2\bar{F}_1)=\tfrac{1}{2}(\bar{B}_1+\bar{B}_2)$$

and
$$\bar{F}_3=\tfrac{1}{4}(\bar{P}_1+\bar{P}_2+2\bar{F}_2)=\tfrac{1}{8}(3\bar{P}_1+3\bar{P}_2+2\bar{F}_1)$$

Corresponding expressions can be found for $\bar{F}_4$ and the means of other types of family.

These relations can, of course, hold only within the limits of accuracy set by the sampling error to which the various mean measurements are subject. When using the relations as tests of conformity with the first criterion of scaling, regard must therefore be paid to the variances of the backcross means, &c. This may be done by putting, for example,

$$A=2\bar{B}_1-\bar{P}_1-\bar{F}_1$$

whereupon
$$V_A=4V_{\bar{B}1}+V_{\bar{P}1}+V_{\bar{F}1}$$

with $V_{\bar{B}1}$ as the variance of the mean measurement of B_1, &c. The standard error of A is then obtained as $\sqrt{V_A}$ and a test of significance is applied by the customary methods.

If the gene effects are additive on the average, those various relations must all hold whatever the situation may be with regard to dominance and linkage. Individual relations may, however, also hold or appear to hold even where additiveness is not the rule. Generally the test based on the F_2 will be expected to be more sensitive than those based on the backcrosses in detecting departures from additiveness, because it will cover a wider range of genotypes and phenotypes. This expectation is on the whole borne out, but there are cases, as we shall see in the next section, when backcross tests reveal non-additiveness which fails to appear in the test using F_2.

The tests of average additiveness may be rendered nugatory by differential viability or fertility in the segregating generations. Coarser tests then become necessary. One general rule may be mentioned as useful in at least some cases. In the absence of certain extreme forms of interaction, $\bar{F}_1$, $\bar{F}_2$, $\bar{F}_3$, &c., must all, apart from error variance, lie on the same side of M, the average of the parents or mid-parent as we may call it, though with diminishing deviations from it. If therefore a scale is obtained upon which $\bar{F}_1$ and $\bar{F}_2$, &c., fall reasonably close to, but on differing sides of, M, their departures must be due largely to the error variation, which differential viability and fertility may be inflating beyond the level assumed in the precise tests of additiveness. In such a case the scale must be regarded as adequate within the limits of the system even though the precise tests show significant discrepancies, for these discrepancies will generally be caused by failure of the genetic assumptions rather than by inadequacy of the scale.

Little comment is required on the tests of conformity with the second criterion of scaling, that of independence of the magnitude of non-heritable variation from the genotype. Two or more genotypes showing different mean measurements are clearly required for the test, and each must be represented by a number of individuals whose variation, due to non-heritable agencies, can be measured. Clones can be used, or the tests can be based on true-breeding lines, or on first crosses between such lines. The variances of true-breeding parents and of their F_1 in an experiment will in fact generally supply the material for a test. The variances of these families may be compared by variance ratios, such as are used in the analysis of variance technique. Significant differences among the variances will show that the scale does not satisfy the criterion.

The variance may fall off with the mean measurement and re-scaling can then usefully be undertaken. Cases are, however,

known where an F_1 intermediate between its parents in mean measurement, has a variance higher or lower than those of both parents. An example of this situation is given in the next section. Where this happens re-scaling by one of the usual simple transformations could not equalize the variance, though a more complex transformation might do so. The causes of such behaviour are, however, likely to be such that a scale which fully satisfies this criterion would not be one on which gene effects are additive. Some compromise is therefore likely to be necessary in such cases.

9 SOME EXAMPLES OF SCALING TESTS

Data on the mean measurements of the two parents, the F_1 and F_2 generations and the backcrosses of the F_1 to the parents have been given by Powers (1941) for six characters in certain tomato crosses. Powers has discussed the adequacy of the scales used in taking the measurements for representation of the characters and for analysis of the variation. In a later paper (1942) he has dealt further with the use of the logarithmic transformation for data on fruit weights. The data on loculus number will serve to illustrate the scaling tests.

The mean numbers of loculi per fruit in the derivatives of four crosses, one of which, Danmark × Johannisfeuer, was grown in the two years 1938 and 1939, are repeated in Table 6. The quantities A, B and C and their variances have been calculated to test the adequacy of the scale in each case, using the formulae

$$A=2\bar{B}_1-\bar{P}_1-\bar{F}_1 \qquad V_A=4V_{\bar{B}1}+V_{\bar{P}1}+V_{\bar{F}1}$$
$$B=2\bar{B}_2-\bar{P}_2-\bar{F}_1 \quad \text{and} \quad V_B=4V_{\bar{B}2}+V_{\bar{P}2}+V_{\bar{F}1}$$
$$C=4\bar{F}_2-2\bar{F}_1-\bar{P}_1-\bar{P}_2 \qquad V_C=16V_{\bar{F}2}+4V_{\bar{F}1}+V_{\bar{P}1}+V_{\bar{P}2}$$

TABLE 6

Mean Number of Loculi per Fruit in the Tomato (Powers, 1941)

	D × J 1938	D × J 1939	J × BB 1939	D × RC 1938	J × RC 1939
$\bar{P}_1$	5·475±0·057	6·183±0·065	9 028±0·084	5·405±0·068	9·028±0·084
$\bar{B}_1$	5·575±0·064	5·898±0·097	7·034±0·162	3·473±0·037	4·356±0·140
$\bar{F}_1$	5·500±0·086	6·051±0·096	6·329±0·111	2·395±0·018	2·517±0·029
$\bar{F}_2$	6·595±0·118	6·826±0·150	6·781±0·208	2·570±0·022	2·886±0·078
$\bar{B}_2$	7·500±0·100	7·344±0·136	5·452±0·077	2·205±0·015	2·183±0·016
$\bar{P}_2$	9·125±0·091	9·028±0·084	6·318±0·069	2·050±0·014	2·034±0·004
A	0·175±0·164	−0·438±0·226	−1·289±0·353	−0·854±0·102	−2·833±0·294
B	0·375±0 236	−0·391±0·300	−1·743±0·202	−0·035±0·038	−0·185±0·043
C	0·780±0·581	−0·009±0·639	−0·880±0·887	−1·965±0·118	−4·552±0·328

$A=2\bar{B}_1-\bar{F}_1-\bar{P}_1$ $\quad B=2\bar{B}_2-\bar{F}_1-\bar{P}_2$ $\quad C=4\bar{F}_2-2\bar{F}_1-\bar{P}_1-\bar{P}_2$

In each case P_1 is the first and P_2 the second variety in the column heading.

D=Danmark, J=Johannisfeuer, BB=Bonny Best, RC=Red Currant.

If the scale is adequate these quantities A, B and C will each equal zero within the limits of sampling error. Powers gives the standard errors of his means and by squaring these the corresponding variances can be found. V_A, V_B and V_C are then determined from the formulae given above. The standard errors of A, B and C, entered in Table 6 have been found as the square roots of the corresponding variances. For example, in the cross Danmark × Johannisfeuer as grown in 1938,

$$A=2\times 5{\cdot}575-5{\cdot}500-5{\cdot}475=11{\cdot}150-10{\cdot}975=0{\cdot}175$$

and $$V_A=(4\times 0{\cdot}064^2)+(0{\cdot}086^2)+(0{\cdot}057^2)=0{\cdot}0270$$

giving a standard error for A of $\sqrt{0{\cdot}0270}$ or 0·164. The values of A, B and C together with their standard errors are also entered for the various crosses in Table 6.

The data from the cross Danmark × Johannisfeuer, whether grown in 1938 or 1939, give insignificant values of A, B and C. Hence the scale used is perfectly adequate for the analysis of the variation in these single sets of data. If no other data were available no suspicion would rest on the scale, because empirically it would be satisfactory. The cross Johannisfeuer × Bonny Best, however, shows significant deviations from zero of both A and B and the scale must be judged inadequate for this set of data in consequence. It may be observed that this difference between the crosses is not due to the second one including a greater range of loculus numbers than the first. The limits are in fact almost the same in the two sets of data as recorded in 1939. The two crosses which include Red Currant both indicate the inadequacy of the scale, the values of A and C being, as would be expected, especially large where the range of loculus numbers is greatest, viz. in the cross Johannisfeuer × Red Currant.

Returning to the cross Danmark × Johannisfeuer, the values of A, B and C are all positive for the 1938 data, but all negative for 1939. Even though neither set is significant of itself, the differences between the values for the two years are worthy of being tested. If A_8 and A_9 are from 1938 and 1938 data respectively we can test the significance of the difference between them by comparing A_8-A_9 with its standard error, found as $\sqrt{V_{A8}+V_{A9}}$. Applying the same calculation also to both B and C, we find

$$A_8-A_9=0{\cdot}613\pm 0{\cdot}279$$

$$B_8-B_9=0{\cdot}766\pm 0{\cdot}382$$

$$C_8-C_9=0{\cdot}789\pm 0{\cdot}864$$

which at least strongly suggests a difference in scale between the two years. Interestingly enough the differences show up more strongly in the backcrosses than they do in F_2, as was also observed in the direct test of Johannisfeuer × Bonny Best.

Thus two conclusions emerge in regard to loculus number. (i) A scale which is adequate for one cross may not be adequate for another which covers the same range of variation, and still less for other crosses covering wider ranges of variation. (ii) A single cross and its descendents may require different scales when grown in two successive years. The difference between years argues that the non-heritable variation is not independent of the genotype, but it does not seem large enough to cause serious difficulty in analysing the variation. It may be that a scale could be found which would prove adequate for all these data. This has, however, not yet been obtained, and the possibility cannot be tested further without resort to the original data, which have not been published. The preponderantly negative values of A, B and C suggest a transformation which would foreshorten the upper end of the scale and Powers tried an approximation to the logarithmic transformation on the data. This, though giving a better fit with additiveness than the untransformed scale, was, he thought, still not fully adequate.

Of the remaining five characters, four, of which two showed heterosis in some crosses, demanded no rescaling. The data on mean weights of individual fruits resembled the results on loculus number, however, in showing some departures which suggest the need for a transformation to foreshorten the upper end of the scale. Powers (1942) has tested the use of log grammes for representing the weights in the cross Danmark by Red Currant. Even after this logarithmic transformation the values of A, B and C differ significantly from zero. In being still negative they show that a more powerful transformation is needed (Table 7).

TABLE 7

Individual Fruit Weight of Tomatoes in Log Grammes (Powers, 1942)
Cross:—Danmark × Red Currant

	P_1	B_1	F_1	F_2	B_2	P_2
Mean	1·6715	1·1619	0·7074	0·6720	0·2870	−0·0534
S.E. of mean	0·0280	0·0093	0·0167	0·0126	0·0159	0·0122
Non-heritable variance	0·0210	—	0·0179	—	—	0·0129

$A = -0{\cdot}0552 \pm 0{\cdot}0376$ $B = -0{\cdot}0801 \pm 0{\cdot}0379$ $C = -0{\cdot}3449 \pm 0{\cdot}0676$

The same conclusion is suggested by the variances of fruit weights due to non-heritable causes, shown by the parents and F_1 generation. These are not equal, but rise linearly with the mean measurement. Powers considers that there is theoretical justification for regarding as suitable a scale on which non-heritable variance is proportional to mean measurement; but, as we have seen, a scale on which these quantities are independent is desirable for analytical purposes. Since the differences on the log scale are not large, there seems a possibility that a transformation which would satisfy the criterion of average additiveness of genic effects, would also satisfy that of independence of non-heritable variation and genotype. Again this cannot be tested in the absence of the original data; but as the departures from satisfaction of the two criteria are small on the log scale, it may well be that the labour of finding a still more satisfactory transformation would not prove to be justified by the extra precision obtained in the analysis of the variation into its components. A compromise between extra precision and economy of labour may be regarded as reasonable for most purposes.

In some cases the use of an approximate scale is unavoidable. An example is afforded by the data on corolla length in a species cross, *Petunia axillaris* × *P. violacea* (Table 8). The measurements were originally taken in millimetres, but it is quite clear from the calculation of C that this scale is not a desirable one. Backcrosses are available and they give values of A and B which also suggested inadequacy of the scale, though the scaling tests based on the backcrosses are here less trustworthy than that based on F_2, owing to the possibility of high selective elimination of certain classes of gamete (Mather and Edwardes, 1943). The inadequacy of the scale is also brought out by the highly unequal variances of the two parents and the F_1.

TABLE 8

Corolla Length in *Petunia* (Mather, unpublished)

	Millimetres		Log measure	
	Mean	Variance	Mean	Variance
P. axillaris	61·28±0·745	35·62	7·871±0·0585	0·224
F_1	35·20±0·122	2·97	5·467±0·0152	0·048
F_2	29·90±0·366	—	4·953±0·1315	—
P. violacea	18·51±0·333	2·38	2·669±0·0800	0·118
C	−30·60±1·692	—	−1·662±0·536	—
Mid-parent	39·89±0·408	—	5·270±0·0496	—

On transforming the data into log measure the scaling criteria are much more nearly satisfied. *C* is still significantly negative, and this might be taken as suggesting the need for a still stronger transformation. The F_1 and F_2 means are, however, an opposite sides of the mid-parent value, so showing, that in regard to this test at least, the scale is adequate. It therefore appears that some agency other than the mere matter of scale is involved. Some differential viability and selective fertilization are to be expected in the offspring of a species cross, and indeed there is evidence of such from the F_2 data themselves (though not to extent observed in the backcrosses by Mather and Edwardes). The significant value of *C* is therefore not to be taken as evidence so much of inadequacy in the scale as of the existence of sources of error in the test which the standard errors, as calculated, do not cover. The log scale is consequently an approximation sufficiently good for the purpose of analysis.

With regard to the parental and F_1 variances, it will be seen from Table 8 that on the log scale the larger parent still has twice the variance of the smaller parent. But the F_1, whose mean is intermediate, has a much lower variance than either. Now the smaller parent *P. violacea* consisted of a clone, while *P. axillaris* was grown from seed. The line of *P. axillaris* was not known to have been highly inbred previously to use in the experiments, and so some of its variation may have been genetic. This coupled with the unexpectedly low variance of F_1 makes it doubtful whether any further adjustment of the scale is worth while. Any simple transformation which made the parental variances more nearly equal must increase the difference between parents and F_1. Any simple adjustment which brought the F_1 variance up to that of *P. violacea* would exaggerate the parental difference. Thus, from the point of view of the variances as well as of the means, the log scale is probably as good as any that is likely to be found.

The low variance of the F_1 families is of some genetical interest. It has generally been supposed that where the non-heritable variance of F_1 differed from the non-heritable variances of the parents, it should exceed them. The present unexpectedly low value of F_1 variance as compared with that of the *P. violacea* clone was observed in both the years in which the plants were grown, and so must be regarded as reasonably well established.

Scaling difficulties due to selective fertilization or differential viability are likely to be especially common with species crosses, but disturbances of the kind produced by these agencies also occur

in varietal crosses. Quisenberry (1926) has recorded grain length in two varieties of oats, Sparrowbill and Victor, and in the F_2 and F_3 generations of reciprocal crosses between them, all plants being grown in 1924. From his data, it appears that the mean length in Sparrowbill is 11·48 mms. and in Victor, 16·36 mms. The F_1 is not available for comparison, but the F_2 means are 14·29 and 14·20 in Sparrowbill × Victor and its reciprocal respectively. The mean of the F_3 means in the former cross is 13·90 and in the latter is 13·77. If we calculate $\bar{F}_3-\frac{1}{2}\bar{F}_2-\frac{1}{4}\bar{P}_1-\frac{1}{4}\bar{P}_2$ we find −0·82 and −1·16 for the two crosses, the expectation being 0. The standard errors of these values are 0·449 and 0·467 respectively. The deviation is significant in the second cross at least. Taking the two crosses together we obtain as the departure from expectation −1·99±0·648, and this must also be judged significant. The scale is therefore apparently suspect. Yet the mid parent is 13·92, which lies between the F_2 and F_3 means. As in the case of the Petunias it must therefore be concluded that the scale is adequate, the discrepancy revealed by the more exacting test being due to slight departures from the simple Mendelian expectations in F_2 and F_3. Such small departures are, however, not likely to prejudice the conclusions obtained from an analysis of the data as they stand.

10. TRANSFORMATION AND RESIDUAL INTERACTION

Transforming a set of data by taking logarithms is equivalent to measuring the character on a scale graduated like a slide rule. In fact, any such transformation is equivalent to the use of a measuring instrument graduated in some appropriate way. It is, however, clearly more expedient in the great majority of cases to take the measurements on a conventional scale and to transform them later, than it is to use a specially graduated instrument. The only possible drawback is a slight distortion of the errors of measurement, for equal errors on, say, a scale of millimetres will become unequal when the data are transformed into log millimetres. This is not likely to be a serious consideration over the ranges of variation ordinarily encountered.

It is essential that the original measurements themselves be transformed individually, before the means, variances and other statistics are calculated. Otherwise the statistics will be distorted, because, to take an example, the log of a mean is not also the mean of the logs (see Mather, 1946*a*). Nor can we test the adequacy of a log scale by finding geometric means of the statistics based on

untransformed measurements. The statistics must be recalculated from the individually transformed measurements.

The transformation to be used in order to adjust the data to a more adequate scale must, in the absence of any theoretical considerations, be a matter of expediency. The log transformation is an obvious choice when the upper end of a scale needs foreshortening. It has also been regarded as especially appropriate on the grounds that growth is more likely to proceed on a log scale than any other. Little if any evidence has, however, been obtained to support this view. Generally the argument has been simply that development must proceed on either an arithmetic or a geometric basis so that if F_1 and F_2 means agree better with the geometric than the arithmetic mean of the parents, the geometric basis must be inferred (see MacArthur and Butler 1938). This argument is wholly unsound as Powers' data show. The fruit weights in his tomatoes demand a scale with even more foreshortening of the upper end than is afforded by the log transformation.

Our chief concern must be to secure a scale permitting the type of analysis which is envisaged. In so far as it is desired to be able to neglect interaction, the scale used must be one upon which this neglect will not vitiate the conclusions reached about other components of variation. If all the genes bear the same relation to each other the choice of an appropriate scale will make all their individual effects additive, and no interaction will remain. Where, however, the interrelations of one pair of genes in action are not the same as those of others, the scale can at best be merely one on which effects are additive on the average. Such residual, though balanced, genic interactions must constitute a source of error variance; but, as Fisher, Immer and Tedin (1932) point out, there is no reason to suppose that they will introduce any bias similar to the metrical bias which arises from an inadequate scale. In other words, residual genic interaction, like non-heritable variation and sampling variation, may lower the precision of the genetic analysis, but it will not be expected to falsify interpretation where valid estimates of error are calculated.

Complementary and epistatic relations, if they occur between polygenes, will have effects which cannot wholly be removed though they may be minimized and balanced one against another by scaling. The extent to which such types of interaction occur in polygenic variation must be explored progressively by experiments where variation in backcrosses is compared with that in F_2 and F_3. No such experiments are available yet, but some evidence is

obtainable from comparisons of variances and covariances in F_2 and F_3 alone. As we shall see later (Section 24), evidence of this kind has failed to reveal any suggestions of genic interaction. It would, however, be unwise to conclude from the little evidence to hand that such types of genic interaction are absent from all polygenic systems.

CHAPTER 4

THE COMPONENTS OF VARIATION

11. DOMINANCE AND POTENCE

VARIATION MEASURED by suitable experiments in a way which satisfies the criteria of scaling can be partitioned into three components. First there is non-heritable variation, resulting from the action of environmental agencies. Some effects of irremovable genic interaction may be included with this. The second component depends on the differences in average character expression associated with the two homozygotes for each of the gene pairs involved. Heritable variation between true breeding strains must always be of this kind and in this sense such variation may be described as *fixable*. The third and final component of variation arises from the differences between the expression of heterozygotes and the average of the two corresponding homozygotes. The magnitude of this last contribution to the variation must depend on the dominance relations of the genes. In so far as such variation cannot be utilized in the selection of true breeding strains, it may be described as *unfixable*.

A method has been developed by Fisher, Immer and Tedin (1932) of determining the contributions of each gene to the fixable and unfixable components of variation, especially as measured by second- and third-degree statistics of the various generations following crossing of true breeding strains. Following these authors, let the average effects of the three genotypes for gene **A-a** on the magnitude of the character in question be

$$\mathbf{AA}\ d_a;\ \mathbf{Aa}\ h_a;\ \mathbf{aa}\ -d_a$$

The designation of one allelomorph by **A** and the other by **a** does not here have the conventional implication with regard to dominance. Similarly for gene **B-b** the three effects will be

$$\mathbf{BB}\ d_b;\ \mathbf{Bb}\ h_b;\ \mathbf{bb}\ -d_b, \text{ and so on.}$$

In all cases d represents an increment in a constant direction along the scale of measurements from the zero point, termed for convenience the + direction, while h may be an increment in either direction, + or −, according to the dominance relation (Fig. 5). With adequate scaling, d and h will have constant average values

over the length of the scale, though irremovable interaction may lead to some variation from these average values at certain points.

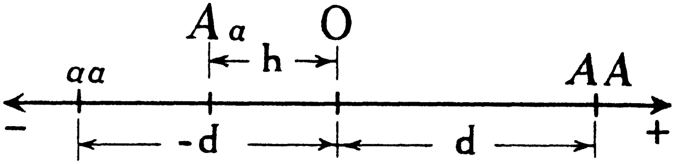


FIG. 5

The d and h increments of the gene **A-a**. Deviations are measured from the point O, mid-way between the homozygotes **Aa** may lie on either side of this point and the sign of h will vary accordingly

A true breeding strain will have an average measurement of

$$S(d_+)-S(d_-)+C$$

where $S(d_+)$ indicates the summed effects of those genes under consideration which are represented by their + allelomorphs, i.e. those adding increments in the + direction, $S(d_-)$ indicates the corresponding sum of effects of genes represented by their − allelomorphs, and C is a constant depending on the actions of genes not under consideration and of non-heritable agencies. If two such strains are grown under comparable conditions their mean measurements will differ by

$$2[S(d_+)-S(d_-)]$$

where only the genes by which they differ are taken into account, and the smaller mean measurement is subtracted from the larger. The mean of the strain means is C, and is independent of the distribution of the genes between the strains. This mid-parent value is the natural zero point from which measurements can be expressed as deviations.

Where all the k genes by which the strains differ are represented in one strain by their + allelomorphs and in the other by their − allelomorphs (i.e. are isodirectionally distributed), the difference between the strain means becomes

$$2S(d_a) \text{ or } 2kd$$

in the special case where $d_a=d_b= \ldots d_k$.

The F_1 between these strains must be heterozygous for all k genes, and when grown under comparable conditions its mean will deviate from the mid-parent by

$$S(h_a) \text{ or } kh$$

in the special case where $h_a=h_b= \ldots h_k$, taking sign into account.

In such a cross the ratio which the deviation of F_1 from the mid-parent bears to half the parental difference is frequently regarded as a measure of average dominance of the genes concerned. We now see that this measure is, in fact.

$$\frac{S(h)}{S(d_+)-S(d_-)}$$

Its use as a measure of average dominance is therefore tantamount to assuming (i) that the genes are isodirectionally distributed and (ii) that all the h increments have the same sign. Neither assumption can be justified, and so it is better to speak of it as the *potence* ratio measuring the relative potence of the gene sets (Wigan, 1944; Mather, 1946*a*). This is made the more desirable by the possibility of measuring the average dominance of the genes in a way to be discussed later.

The potence ratio can in fact theoretically take any value between 0 and ∞. This is true even with the restriction that $h \leqslant d$ for each gene, i.e. that for any gene the heterozygote never falls outside the range delimited by the two homozygotes. If the h increments are balanced, in the sense that the sum of h increments of the genes whose heterozygotes resemble the + homozygote more than the − homozygote (+ allelomorph dominant) equals the summed h increments of the genes to which the opposite (− allelomorph dominant) applies, then

$$S(h)=0$$

and the F_1 will fall on the mid-parent. Potence is zero, no matter what the average dominance may be. Similarly where the parents do not differ in mean measurement, i.e. $S(d_+)=S(d_-)$, potence must be ∞ with any condition other than $S(h)=0$. Heterosis will follow wherever

$$S(h)>[S(d_+)-S(d_-)]$$

even though $d \geqslant h$ for all genes.

In short, observable potence of sets of genes indicates dominance of the individual genes preponderantly in the same direction; but zero potence does not of necessity indicate absence of dominance. Equally heterosis indicates dominance, $h \neq 0$, but not of necessity super dominance $h>d$.

12. VARIATION IN F_2 AND F_3

The variation of the measurements of true breeding parents and their F_1 must be exclusively non-heritable. The variances of these

measurements consequently afford estimates of the non-heritable contribution to the variances of later generations, such as F_2, where heritable components will also be present. With no differential fertilization or viability the constitution of F_2 in respect of a segregating gene **A-a** will be $\frac{1}{4}$**AA**; $\frac{1}{2}$ **Aa**; $\frac{1}{4}$**aa**, and this gene will contribute increments of d_a; h_a; $-d_a$ to the measurements of plants in the three classes respectively. The contribution of **A-a** to the F_2 mean, expressed as a deviation from the mid-parent, will therefore be $\frac{1}{2}h_a$ since the contributions of **AA** and **aa** cancel one another. Taking all k genes into account the F_2 mean will be $\frac{1}{2}S(h_a)$, i.e. half the F_1 mean.

The contribution of **A-a** to the sum of squares of deviations from the mid-parent will be $\frac{1}{4}d_a^2+\frac{1}{2}h_a^2+\frac{1}{4}(-d_a)^2$ so that the contribution to the sum of squares of deviations from the F_2 mean becomes $\frac{1}{2}d_a^2+\frac{1}{2}h_a^2-(\frac{1}{2}h_a)^2$ or $\frac{1}{2}d_a^2+\frac{1}{4}h_a^2$. Since the frequencies of the three genotypes total unity, the contribution to the sum of squares is also the contribution to the mean square measuring heritable variance.

Provided that the genes neither interact nor are linked, the total heritable variance given by k genes in F_2 will be the sum of the k individual contributions, viz.

$$\tfrac{1}{2}S(d_a^2)+\tfrac{1}{4}S(h_a^2)$$

The fixable and unfixable heritable components are separable in this variance, for d measures the difference of effect between homozygotes, while h is the measurement characterizing the heterozygote.

In F_3, the families from **AA** and **aa** F_2 individuals will be wholly **AA** and **aa** respectively, while those from heterozygous F_2 individuals will repeat the F_2 segregation. The means of families from **AA**, **Aa**, and **aa** parents will therefore be d_a, $\frac{1}{2}h_a$, and $-d_a$ in respect of this gene. The grand mean of these means must be $\frac{1}{4}h_a$ from the mid-parent, and the contribution of **A-a** to the variance of F_3 means will be

$$\tfrac{1}{4}d_a^2+\tfrac{1}{2}(\tfrac{1}{2}h_a)^2+\tfrac{1}{4}(-d_a)^2-(\tfrac{1}{4}h_a)^2 \quad \text{or} \quad \tfrac{1}{2}d_a^2+\tfrac{1}{16}h_a^2$$

Since the situation will be similar for other genes, the general mean of all F_3's will be $\frac{1}{4}S(h_a)$ and the total heritable variance of F_3 means will be

$$\tfrac{1}{2}S(d_a^2)+\tfrac{1}{16}S(h_a^2)$$

It can similarly be shown that the covariance of F_3 mean with its F_2 parent's measurement is $\frac{1}{2}S(d_a^2)+\frac{1}{8}S(h_a^2)$ and the mean variance of F_3 families is $\frac{1}{4}S(d_a^2)+\frac{1}{8}S(h_a^2)$.

These results are given by Fisher, Immer and Tedin together with similar ones for progenies of the third generation, which are

obtained by intercrossing pairs of plants taken at random from F_2 (biparental progenies) or by exposing one F_2 plant as mother to pollination by the pollen of the F_2 as a whole (maternal progenies). In each case the formula is expressible in two parts, one depending on $S(d_a^2)$ which is contributed by the fixable variation, and the other on $S(h_a^2)$ contributed by the unfixable variation. These components may be conveniently denoted by D and H respectively so that the F_2 variance, for example, becomes $\frac{1}{2}D+\frac{1}{4}H$.

These various formulae have been collected into Table 9. The variances must contain a non-heritable component in addition to the two heritable portions. This is denoted by E in the table. Covariances will, of course, be free from non-heritable components since the pairs of measurements, which give the cross-products from which covariances are calculated, are as likely to be affected in opposite ways as the same way by non-heritable agencies.

TABLE 9

Components of Variation in F_2 and its Derivatives

V_{F2}	—F_2 variance	$\frac{1}{2}D+\frac{1}{4}H+E$
V_{F3}	—Variance of means of F_3 progenies	$\frac{1}{2}D+\frac{1}{16}H+E$
$W_{F2/F3}$	—Covariance of F_3 mean and F_2 parental measurement	$\frac{1}{2}D+\frac{1}{8}H$
$\bar{V}_{F3}$	—Mean variance of F_3 progenies	$\frac{1}{4}D+\frac{1}{8}H+E$
$V_{\overline{BIP}}$	—Variance of means of biparental progenies	$\frac{1}{4}D+\frac{1}{16}H+E$
$W_{F2/BIP}$	—Covariance of biparental mean and F_2 parental measurement	$\frac{1}{4}D$
$\bar{V}_{BIP}$	—Mean variance of biparental progenies	$\frac{1}{4}D+\frac{3}{16}H+E$
$V_{\overline{MAT}}$	—Variance of means of maternal progenies	$\frac{1}{8}D+E$
$\bar{V}_{MAT}$	—Mean variance of maternal progenies	$\frac{3}{8}D+\frac{1}{4}H+E$

where $D=S(d^2)$
and $H=S(h^2)$
E is not constant (see in text)

With linkage D and H are no longer related to d_a and h_a, &c., in the way shown. They remain constant, however, over the range of formulae except for $\bar{V}_{F3}$, $\bar{V}_{BIP}$ and $\bar{V}_{MAT}$ (see Section 16).

Where estimates of a number of the variances and covariances shown in Table 9 are available, D and H can be estimated separately; and, what is more, their error variances can also be found (see Section 13). It is then possible to obtain an estimate of $\frac{H}{D}$ which affords a measure of the average dominance of the genes concerned. In fact, if we care to assume that $d_a=d_b \ldots =d_k$ and $h_a=h_b \ldots =h_k$, sign being neglected, a direct measure is available for $\frac{h}{d}=\sqrt{\frac{H}{D}}$. This assumption will doubtless seldom be true, but the

ratio $\frac{H}{D}$ will serve to measure dominance sufficiently well to enable the composite nature of the potence relation to be demonstrated and appreciated.

The effects of linkage on these variances and covariances will be considered in the next chapter, but a word about irremovable genic interaction is necessary here. With such interaction the d and h increments depending on a given gene will not be constant over all the genotypes. In particular it can be shown for particular types of irremovable interaction, epistasis, &c., that not only are the relations $D=S(d^2)$ and $H=S(h^2)$ no longer strictly true, but also that the magnitude of the disturbance will vary between the different variances and covariances. The error variance of D and H as estimated from these various statistics, must reflect these interaction effects as well as the outcome of non-heritable agencies. Interaction may therefore distort the estimates of D and H somewhat, but the simultaneous inflation of their error variances will introduce a due measure of caution into the interpretation of their relations. The detection of such residual interactions from their effects on D and H will be discussed later.

Before leaving the F_2 generation and its derivatives, it must be mentioned that Fisher, Immer and Tedin have derived the formulae for certain third degree statistics. These are all compounded of two components, viz. $S(hd^2)$ and $S(h^3)$, which are denoted by F and G respectively in Table 10, where the formulae

TABLE 10

Contributions of d and h to Third-Degree Statistics

Skewness of F_2	$-\frac{3}{4}F$
Mean skewness of F_3 progenies	$-\frac{3}{8}F$
Covariance of means and variances of F_3 progenies	$\frac{1}{16}F+\frac{1}{32}G$
Covariance of F_2 parental measurement and F_3 variance	$\frac{1}{8}F+\frac{1}{16}G$
Skewness of F_3 means	$-\frac{3}{8}F$
Mean skewness of biparental progenies	$-\frac{3}{16}F$
Covariance of means and variances of biparental progenies	$-\frac{1}{8}F$
Covariance of F_2 parental measurements and biparental variance	$-\frac{1}{16}F+\frac{1}{32}G$
Covariance of biparental means and biparental product	$-\frac{1}{8}F$
Skewness of biparental means	$-\frac{3}{16}F$
Mean skewness of maternal progenies	$-\frac{3}{8}F$
Covariance of mean and variance of maternal progenies	$-\frac{1}{8}F$
Covariance of F_2 parental measurement and maternal variance	$-\frac{3}{16}F$
Skewness of mean of maternal progenies	0

Where $F=S(hd^2)$

and $G=S(h^3)$

Skewness is measured by $\frac{n}{(n-1)(n-2)}S(x-\bar{x})^3$

are reproduced. It should be observed that in these third-degree statistics the sign of h is taken into account, in contrast to the second degree statistics of Table 9 where h^2 is used exclusively, sign being removed from account in consequence. Thus with balanced dominance F and G will approximate to zero, even though h_a, h_b, &c., all have values departing from 0. The balancing effects of sign will not, of course, operate in H, which will therefore reflect the existence of dominance even where potence is zero.

13. PARTITIONING THE VARIATION OF F_2 AND ITS DERIVATIVES

The use of the various second-degree statistics in estimating the contributions made by non-heritable, fixable heritable and unfixable heritable variations may be illustrated by Quisenberry's (1926) data on grain length in oats, to which reference has already been made. The partitioning operation in this case is made somewhat more complex than usual, because the various families were not grown in accordance with modern principles of experimental design. This disadvantage is, however, outweighed by the wealth of data which this author presents.

The varieties Sparrowbill (S) and Victor (V) were crossed reciprocally in 1922, the F_1's being winter grown in the greenhouse. F_2's were raised in 1923, and from them 150 F_3's were obtained, 75 from each of the reciprocal crosses. These were grown together with the parent varieties and further F_2's in 1924. The 189 F_2 plants of S × V and the 224 F_2 plants of V × S, were not further subdivided, but the 1290 plants of S and the 1291 plants of V were each divided into 36 groups of average sizes 35·83 and 35·86 plants respectively. The 75 F_3 families from S × V and the 75 F_3's from V × S contained on the average 32·47 and 32·31 plants respectively.

Now the variance of plants within the same group was 0·2844 in S and 0·3356 in V. The variance of plants from different groups was 1·3583 in S and 1·6741 in V, so that position is clearly affecting the grain length. Since the parental groups and F_3 families approximated closely in average size, the variance within parental groups may be taken as a measure of the non-heritable component of mean variance of F_3 progenies. Equally the variance of F_3 means will contain a non-heritable component related to the inter-group variance of the parents. The actual measure of this non-heritable component in the variance of F_3 means will be given by the ratio of the inter-group variance to the number of plants in each F_3 progeny; but since this number varied, the harmonic mean

of the number of plants in the 150 F_3's must be used. The inter-group variance of the parents is therefore divided by 30·6413 to obtain the estimate of the non-heritable component of variance of F_3 means. The S parent gives in this way $\frac{1·3583}{30·6413}$, i.e. 0·0443 and the V parent 0·0546.

The reciprocal F_2's of 189 and 224 plants were not divided into groups and so require still a third estimate of non-heritable variation. The most reasonable estimate of this non-heritable component of F_2 variance can be found from the gross variance (pooled intra- and inter-group) of the parents. This is 0·3135 for S and 0·3719 for V.

The F_2 variance (V_{F2}), variance of F_3 means ($V_{\bar{F}3}$), covariance of F_3 means and F_2 plant values ($W_{F2/F3}$), and mean variance of F_3's ($\bar{V}_{F3}$) are set out together with the estimates of the three non-heritable components in Table 11. Since duplicate estimates of each of the three non-heritable components are available, one set from the variances of each parent, and since F_2's and F_3's are equally available from the two reciprocal crosses, duplicate values appear in each line of the table. The averages of the duplicates may be used in partitioning the variation, the differences between duplicates then being available for finding the standard errors of the estimates of the various components of variation, as we shall see later.

TABLE 11

Quisenberry's Data on Grain Length in Oats

Mean Length in Millimetres	Parents	S 11·484
		V 16·361
	F_2	S × V 14·291
		V × S 14·201
	F_3	S × V 13·901
		V × S 13·771

		Observed: S or S × V	Observed: V or V × S	Observed: Mean	Expected	Deviations	
V_{F2}	$\frac{1}{2}D+\frac{1}{4}H+E_1$	1·1968	1·4348	1·3158	1·2932	−0·0964	0·1416
$V_{\bar{F}3}$	$\frac{1}{2}D+\frac{1}{16}H+E_2$	0·8523	0·9095	0·8809	0·8289	0·0234	0·0806
$W_{F2/F3}$	$\frac{1}{2}D+\frac{1}{8}H$	0·6614	0·7707	0·7161	0·7942	−0·1328	−0·0235
$\bar{V}_{F3}$	$\frac{1}{4}D+\frac{1}{8}H+E_3$	0·8069	0·7687	0·7878	0·7809	0·0260	−0·0122
Non-heritable components: in F_2	E_1	0·3135	0·3719	0·3427	0·3653	−0·0518	0·0066
Non-heritable components: in F_3 mean	E_2	0·0443	0·0546	0·0495	0·1015	−0·0572	−0·0469
Non-heritable components: in mean variance of F_3	E_3	0·2844	0·3356	0·3100	0·3169	−0·0325	0·0187

Five components of variation are involved, as shown by the expectation of Table 11, viz. D, H and the three non-heritable items E_1–E_3. Seven equations are, however, available for their estimation from the statistics in the table, which must therefore be undertaken by a least squares technique. The various statistics, V_{F2}, &c., are not known with equal precision and so should be weighted if a rigorous least squares analysis is desired. The extra labour of weighting is, however, hardly likely to be justified by additional rigour obtained, and so no weighting will be attempted. A further advantage of unweighted analysis is that, as we shall see, the matrices developed for the analysis of any one experiment can be used for all others of the same design. This would be impossible if weights were introduced.

The seven basic equations, one from each line of the table, are

$$V_{F2}=\tfrac{1}{2}D+\tfrac{1}{4}H+E_1=1{\cdot}3158$$
$$V_{\overline{F3}}=\tfrac{1}{2}D+\tfrac{1}{16}H+E_2=0{\cdot}8809$$
$$W_{F2/F3}=\tfrac{1}{2}D+\tfrac{1}{8}H=0{\cdot}7161$$
$$\bar{V}_{F3}=\tfrac{1}{4}D+\tfrac{1}{8}H+E_3=0{\cdot}7878$$
$$\text{Direct estimates}\begin{cases}E_1=0{\cdot}3427\\ E_2=0{\cdot}0495\\ E_3=0{\cdot}3100\end{cases}$$

These may be combined to give five equations yielding least squares estimates of the five components of variation as follows. Each of the seven equations is multiplied through by the coefficient of D which it contains, and the seven are then summed. Where D does not appear the equation is omitted. Then we have

$$\begin{array}{rl}
 & \tfrac{1}{4}D+\tfrac{1}{8}H+\tfrac{1}{2}E_1 \qquad\qquad =0{\cdot}65790\\
 & \tfrac{1}{4}D+\tfrac{1}{32}H+\quad \tfrac{1}{2}E_2 \qquad =0{\cdot}44045\\
 & \tfrac{1}{4}D+\tfrac{1}{16}H \qquad\qquad\qquad =0{\cdot}35805\\
 & \tfrac{1}{16}D+\tfrac{1}{32}H \qquad\qquad +\tfrac{1}{4}E_3=0{\cdot}19695\\
\hline
\text{Total} & \tfrac{13}{16}D+\tfrac{1}{4}H+\tfrac{1}{2}E_1+\tfrac{1}{2}E_2+\tfrac{1}{4}E_3=1{\cdot}65335
\end{array}$$

The four further equations are found in the same way using the coefficients of H, E_1, E_2 and E_3 as multipliers in turn. The solution of these five simultaneous equations gives the desired estimates of D, &c.

The left sides of the equations are concerned only with D, H and E_1, E_2, and E_3: they will be the same for all experiments of this kind. The right sides of these equations are derived from the values of V_{F2}, &c., observed experimentally, and the solution of the five

equations as they stand can refer only to the experiment in question. Other similar experiments may, however, be analysed using the same solution if the right sides of the equations are replaced successively by 1, 0, 0, 0, 0; 0, 1, 0, 0, 0, and so on, to give five sets of five equations in the way Fisher (1946) recommends for multiple regression analyses. On doing this the equations become

$$\begin{array}{lllll} 0{\cdot}812500D+0{\cdot}250000H+0{\cdot}500000E_1+0{\cdot}500000E_2+0{\cdot}250000E_3 & =1, 0, 0, 0, 0 \\ 0{\cdot}250000D+0{\cdot}097656H+0{\cdot}250000E_1+0{\cdot}062500E_2+0{\cdot}125000E_3 & =0, 1, 0, 0, 0 \\ 0{\cdot}500000D+0{\cdot}250000H+2{\cdot}000000E_1 & =0, 0, 1, 0, 0 \\ 0{\cdot}500000D+0{\cdot}062500H \qquad\qquad +2{\cdot}000000E_2 & =0, 0, 0, 1, 0 \\ 0{\cdot}250000D+0{\cdot}125000H \qquad\qquad\qquad\qquad +2{\cdot}000000E_3 & =0, 0, 0, 0, 1 \end{array}$$

On solving these five sets of equations we obtain a matrix of multipliers of which c_{DD} is the value of D in the first of the five sets, and c_{DH}, c_{D1}, c_{D2}, and c_{D3} the value of D in the second, third, fourth and fifth sets. Similarly c_{HD}, c_{HH}, c_{H1}, c_{H2} and c_{H3} are the values of H in the five sets, and so on. This matrix turns out to be as shown in Table 12.

TABLE 12

From equation set	D		H		E_1		E_2		E_3	
1	c_{DD}	9·978498	c_{HD}	−27·526894	c_{1D}	0·946237	c_{2D}	−1·634409	c_{3D}	0·473119
2	c_{DH}	−27·526894	c_{HH}	93·591441	c_{1H}	−4·817207	c_{2H}	3·956991	c_{3H}	−2·408603
3	c_{D1}	0·946237	c_{H1}	−4·817207	c_{11}	0·865592	c_{21}	−0·086022	c_{31}	0·182796
4	c_{D2}	−1·634409	c_{H2}	3·956991	c_{12}	−0·086022	c_{22}	0·784946	c_{32}	−0·043011
5	c_{D3}	0·473119	c_{H3}	−2·408603	c_{13}	0·182796	c_{23}	−0·043011	c_{33}	0·591398

It will be observed that as $c_{DH}=c_{HD}$, &c. the matrix is symmetrical about one diagonal.

Now in Quisenberry's experiment, as we have already seen, the sum of the F_2 variance, variance of F_3 means, &c., each multiplied by the corresponding coefficient of D, is 1·6534. This we may denote as $S(Dy)$. Similarly from Table 12

$$\begin{aligned} S(Hy)&=0{\cdot}5720\\ S(E_1y)&=1{\cdot}6585\\ S(E_2y)&=0{\cdot}9304\\ S(E_3y)&=1{\cdot}0978 \end{aligned}$$

The least squares estimate of D in this experiment is then found from the D column of Table 12 as

$$\begin{aligned} D&=c_{DD}S(Dy)+c_{DH}S(Hy)+c_{D1}S(E_1y)+c_{D2}S(E_2y)+c_{D3}S(E_3y)\\ &=(9{\cdot}978498\times1{\cdot}6534)+(-27{\cdot}526894\times0{\cdot}5720)+\ldots\\ &=1{\cdot}3211 \end{aligned}$$

H is found from the H column of the table as

$$H = c_{HD}S(Dy) + c_{HH}S(Hy) + c_{H1}S(E_1y) + c_{H2}S(E_2y) + c_{H3}S(E_3y)$$
$$= 1{\cdot}0694$$

Similarly from the remaining columns

$$E_1 = 0{\cdot}3653, \quad E_2 = 0{\cdot}1015 \quad \text{and} \quad E_3 = 0{\cdot}3169$$

On substituting these estimates for D, &c., we find as the expected value of V_{F2}

$$\tfrac{1}{2}D + \tfrac{1}{4}H + E_1 = \tfrac{1}{2}(1{\cdot}3211) + \tfrac{1}{4}(1{\cdot}0694) + 0{\cdot}3653 = 1{\cdot}2932$$

This and the remaining expectations, found in the same way, are given in Table 11 (see Fig. 6). The two values for V_{F2}, found

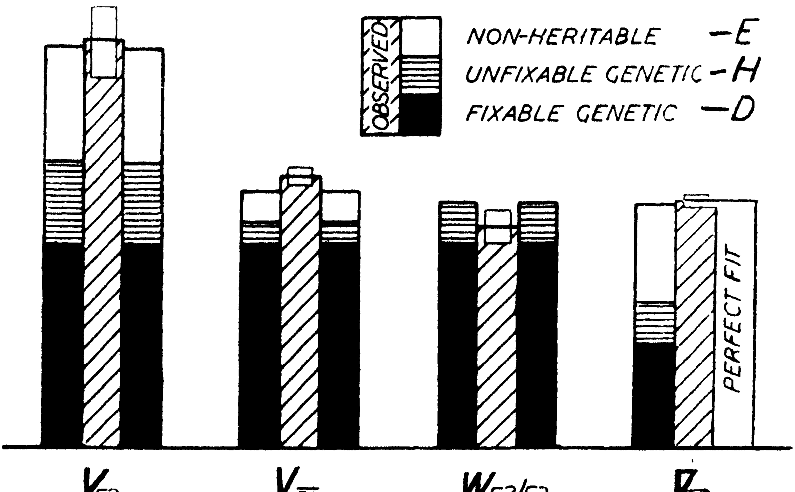


FIG. 6

OATS : GRAIN LENGTH VARIATION

The partition of variability in Quisenberry's (1926) data on grain length in oats. The centre columns show the mean value observed for each statistic, the difference between the two replicates of the experiment being indicated by the box at the top The left columns show the values expected for each statistic, and the composition of these values, when D and H are estimated assuming no linkage The right columns show the same when D and H are estimated making allowance for linkage by assuming a perfect fit in $\bar{V}_{F3}$. The results give no evidence of linkage

from the reciprocal crosses, deviate by $-0{\cdot}0964$ and $0{\cdot}1416$ from the expected value. There are 14 such deviations, two from each line of the table. On squaring and summing, $0{\cdot}065005$ is obtained as the sum of squares of these deviations from expectation. This corresponds to 9 degrees of freedom, for of the 14, contributed 1 by

each deviation,* 5 have been sacrificed in estimating the 5 parameters D–E_3. The mean square deviation (V) is therefore $\frac{0{\cdot}065005}{9}$ or 0·007223.

Now the variance V_D of our estimate of D is given by the relation

$$V_D = \tfrac{1}{2} V c_{DD} = \tfrac{1}{2}(0{\cdot}007223 \times 9{\cdot}978498)$$
$$= 0{\cdot}03603$$

the factor of $\frac{1}{2}$ being introduced by the use of mean values of V_{F_2}, &c., from duplicate observations. The standard error of D is $\sqrt{V_D}$ or 0·1898.

Similarly $s_H = \sqrt{V_H} = \sqrt{\tfrac{1}{2} V . c_{HH}} = 0{\cdot}5814$

$s_1 = 0{\cdot}0559 \quad s_2 = 0{\cdot}0532 \quad s_3 = 0{\cdot}0462$

and the components of variation in this experiment are

$$D = 1{\cdot}3211 \pm 0{\cdot}1898$$
$$H = 1{\cdot}0694 \pm 0{\cdot}5814$$
$$E_1 = 0{\cdot}3653 \pm 0{\cdot}0559$$
$$E_2 = 0{\cdot}1015 \pm 0{\cdot}0532$$
$$E_3 = 0{\cdot}3169 \pm 0{\cdot}0462$$

Since H nearly equals D it would appear that there is a marked degree of dominance, but the high value of s_H makes this conclusion somewhat unreliable ($t_{[14]} = 1{\cdot}84$; P = 0·10–0·05). The approximation of the F_1, F_2 and F_3 means to the mid-parent in Quisenberry's data may therefore be due either to an absence of dominance or, perhaps more likely, to balance of dominance in the two directions. The experiment is not fully discriminative on this point, and indeed F_2 and F_3 data by themselves can seldom be expected to give a very precise value of H owing to the low coefficients of H in the expectations. In backcross data, as we shall see in the next section, the coefficients are higher, with the result that more reliable values of H are to be expected from them.

* The direct estimate of E_1 is partially correlated with those of E_2 and E_3. There are not, therefore, fully 14 initial degrees of freedom, but there must be at least 12. The assumption of 14 leads, however, to no false conclusions, though the standard errors of D, H, &c., are somewhat under-estimated. In adequately designed experiments, such as that with barley (Section 18), this difficulty will not arise.

Certain third-degree statistics were also calculated from these oat data. The skewnesses of the two F_2's were 0·7889 and 0·2791, of which the former seems significantly large, though the latter does not. The mean skewnesses of F_3 from the two crosses were 0·001255 and 0·001171. These are expected to be half the F_2 skewnesses if attributable to genetic causes. It is therefore clear that no reliance can be placed in the apparently significant single F_2 value. The covariances of mean and variance of F_3 were 0·0628 and 0·0546 in the two reciprocals, and the covariances of F_2 parent value and F_3 variance were 0·0467 and 0·0490. Thus none of the third-degree statistics may be regarded as departing from 0 for genetical reasons—a state of affairs which, like the approximation of the F_1, F_2 and F_3 means to the mid-parent, would follow either from the absence of dominance (i.e. $h=0$ for each gene) or from the dominance of increasing (+) allelomorphs in some genes being balanced by dominance of the decreasing (−) allelomorphs in others (i.e. h being + and − in balanced proportions). In either case F and G (see Table 10) are expected to be 0.

The matrix of c multipliers calculated for the oat experiment can be used in partitioning the variation of any set of results from an experiment of that kind. The values of $S(Dy)$, &c., are found from the observations, and on combining with the appropriate columns of Table 12 they give estimates of D, &c. In an experiment constituted differently from that of the oats however, a different c matrix is needed. A more common type of experiment may be illustrated by the *Petunia* species cross already mentioned in Section 9. In this case parental, F_1, F_2 and F_3 families were all much of the same size, though their sizes varied somewhat on account of the varying viability of the seed from which the different families were grown. The non-heritable components of variation in F_2 and the F_3's may, however, be taken as the same, as opposed to the situation with the oats where they could not be so regarded. An estimate of this component may be obtained from the variance of parent and F_1 families. These, as we have seen in Section 9, differed somewhat, that of F_1 being the smallest. A reasonable estimate of the non-heritable component in F_2 and F_3's may be found as the mean of the three variances from the two parents and the F_1. This is $\frac{1}{3}(0{\cdot}2243+0{\cdot}0483+0{\cdot}1177)=0{\cdot}1301$ (see Table 8). The numbers of plants in the 18 utilizable F_3 families varied round a harmonic mean of 9·259, and so the non-heritable component of variance of F_3 means may be estimated as $\frac{0{\cdot}1301}{9{\cdot}259}$ or

0·0141.* These estimates are set out together with the F_2 and F_3 variances and covariances in Table 13.

TABLE 13

Corolla Length in *Petunia*

Mean Length in log measure	Parents	
	P. axillaris	7·871
	P. violacea	2·669
	F_1	5·467
	F_2	4·953

		Observed	Expected	Deviation
V_{F2}	$\frac{1}{2}D+\frac{1}{4}H+E_1$	0·5249	0·4963	0·0286
$V_{\overline{F3}}$	$\frac{1}{2}D+\frac{1}{16}H+E_2$	0·4250	0·4022	0·0228
$W_{F2/F3}$	$\frac{1}{2}D+\frac{1}{8}H$	0·3331	0·3674	−0·0343
$\overline{V}_{F3}$	$\frac{1}{4}D+\frac{1}{8}H+E_1$	0·2762	0·3104	−0·0342
Non-heritable components				
Single plants	E_1	0·1301	0·1244	0·0057
Means of families	E_2	0·0141	0·0370	−0·0229

Since there are only 4 parameters involved, D, H, E_1 and E_2, there will be only four least squares equations for their estimation. These are found from the table in a way exactly analogous with that used for the oats. The equations whose solutions give the c matrix are then

$$0{\cdot}812500D+0{\cdot}250000H+0{\cdot}750000E_1+0{\cdot}500000E_2=1,\ 0,\ 0,\ 0$$
$$0{\cdot}250000D+0{\cdot}097656H+0{\cdot}375000E_1+0{\cdot}062500E_2=0,\ 1,\ 0,\ 0$$
$$0{\cdot}750000D+0{\cdot}375000H+3{\cdot}000000E_1=0,\ 0,\ 1,\ 0$$
$$0{\cdot}500000D+0{\cdot}062500H+2{\cdot}000000E_2=0,\ 0,\ 0,\ 1$$

and the c matrix itself appears as shown in Table 14.

TABLE 14

D		H		E_1		E_2	
c_{DD}	10·526316	c_{HD}	−30·315789	c_{1D}	1·157895	c_{2D}	−1·684211
c_{DH}	−30·315789	c_{HH}	107·789473	c_{1H}	−5·894737	c_{2H}	4·210526
c_{D1}	1·157895	c_{H1}	−5·894737	c_{11}	0·780702	c_{21}	−0·105263
c_{D2}	−1·684211	c_{H2}	4·210526	c_{12}	−0·105263	c_{22}	0·789474

* The direct estimates of E_1 and E_2 are thus correlated, but the calculation is made as if they were not, in order to illustrate the procedure and matrix appropriate to an adequately designed experiment where these estimates would be independent.

Then with

$$S(Dy)=0{\cdot}7106,\ S(Hy)=0{\cdot}2340,\ S(E_1y)=0{\cdot}9312,\ S(E_2y)=0{\cdot}4391,$$
$$D=0{\cdot}7258\pm0{\cdot}1494,\ H=0{\cdot}0361\pm0{\cdot}4781,$$
$$E_1=0{\cdot}1244\pm0{\cdot}0407,\ E_2=0{\cdot}0370\pm0{\cdot}0409.$$

The values expected for V_{F2}, &c., found from these estimates of D, H, E_1 and E_2 are given in Table 13 and the sum of squares of deviations of observed from expected values is 0·0042408. Since there are 6 quantities observed and 4 parameters fitted, 2 degrees of freedom will remain for this sum of squares so that the mean square becomes 0·0021204. The standard errors of D . . . E_2 given above are then found from this mean square using c_{DD}, &c., as multipliers before taking the square root, in the way already described for the oat analysis. No factor of $\frac{1}{2}$ is used in calculating these variances as each of the statistics, V_{F2}, &c., is a unique observation.

Again there is no evidence of dominance. Indeed, with a standard error of 0·4781, evidence of dominance must be hard to obtain. It may be remarked, too, that if estimates of the non-heritable components are derived by combining the parental and F_1 variances in ways other than that used, the estimate of D is changed but little. That of H is, however, markedly altered, sometimes even being negative, though not of course significantly so.

With most animals, F_3 families are unobtainable. Instead, biparental progenies are available from pair matings between males and females taken at random from the F_2. The c matrix in such a case must of course differ from those used above. The use of biparental progenies may be illustrated by the inheritance of number of abdominal chaetae in a cross between the O and B stocks of *Drosophila melanogaster* (Mather, 1941) already mentioned in Section 2. The means of the parents and later generations differ by relatively few chaetae (Table 15).

The flies were raised in the customary half-pint bottles, there being five such cultures of each parent and of the F_1. Twelve F_2 cultures were raised, six from each reciprocal cross, and there were thirty-one biparental progenies of the third generation, each in its own bottle. V_{F2} was found using the deviations from the bottle means, and so its non-heritable component must be that occurring within bottles. The same will be true of the mean variance ($\dot{V}_{BIP}$) of the thirty-one biparental progenies since each was raised in a single bottle. This non-heritable component (E_1) was estimated by pooling the variances within bottles of the parents and F_1. The

variance of biparental means ($V_{\overline{BIP}}$), on the other hand, must be a variance between bottles and its non-heritable component was therefore estimated as the pooled variance between bottle means of parents and F_1, round the parental and F_1 means respectively. Males have fewer chaetae than females but seem to show the same variances. All deviations were therefore calculated from the appropriate sex means, but were pooled in finding the variances and covariances. Sex linkage is a potential source of disturbance. Since, however, the Y chromosome appears to be polygenically active like the X (Mather, 1944), it was not thought likely that sex linkage could distort the results seriously, provided male and female deviations were pooled in the calculations. In any case these data will serve to illustrate the use of biparental progenies.

TABLE 15

Abdominal Chaetae Number in *Drosophila*

			Males	Females
Mean Numbers	Parents	B	36·06	43·48
		O	39·88	44·59
		F_1	37·80	43·43
		F_2	38·35	44·20
		F_3	38·54	44·55

		Observed	Expected	Deviation
V_{F2}	$\frac{1}{2}D+\frac{1}{4}H+E_1$	6·6214	6·9012	−0·2798
$V_{\overline{BIP}}$	$\frac{1}{4}D+\frac{1}{16}H+E_2$	1·2518	1·3130	−0·0612
$W_{F2/BIP}$	$\frac{1}{4}D$	0·4227	0·1956	0·2271
$\bar{V}_{BIP}$	$\frac{1}{4}D+\frac{3}{16}H+E_1$	7·1377	6·7442	0·3935
Non heritable components				
Within bottles	E_1	6·5505	6·6642	−0·1137
Between bottles	E_2	1·2172	1·1560	0·0612

The results, together with the expectations in terms of D and H, are set out in Table 15. The equations whose solutions give the c matrix are then found as

$$0{\cdot}437500D+0{\cdot}187500H+0{\cdot}750000E_1+0{\cdot}250000E_2=1, 0, 0, 0$$
$$0{\cdot}187500D+0{\cdot}101563H+0{\cdot}437500E_1+0{\cdot}062500E_2=0, 1, 0, 0$$
$$0{\cdot}750000D+0{\cdot}437500H+3{\cdot}000000E_1=0, 0, 1, 0$$
$$0{\cdot}250000D+0{\cdot}062500H+2{\cdot}000000E_2=0, 0, 0, 1$$

giving the matrix shown in Table 16.

TABLE 16

D		*H*		E_1		E_2	
c_{DD}	12·394372	c_{HD}	−24·338047	c_{1D}	0·450705	c_{2D}	−0·788733
c_{DH}	−24·338047	c_{HH}	75·718367	c_{1H}	−4·957750	c_{2H}	0·676057
c_{D1}	0·450705	c_{H1}	−4·957750	c_{11}	0·943662	c_{21}	0·098592
c_{D2}	−0·788733	c_{H2}	0·676057	c_{12}	0·098592	c_{22}	0·577465

Further

$S(Dy)=5{\cdot}5138$, $S(Hy)=3{\cdot}0719$, $S(E_1y)=20{\cdot}3096$, $S(E_2y)=2{\cdot}4690$,

whence $D=0{\cdot}7823\pm1{\cdot}3751$, $H=-0{\cdot}6166\pm3{\cdot}3988$, $E_1=6{\cdot}6642\pm0{\cdot}3794$, $E_2=1{\cdot}1560\pm0{\cdot}2968$. The sum of squares of deviations, for 2 degrees of freedom as with the Petunias, turns out to be 0·305123, from which the standard errors of D . . . E_2 are found using the appropriate multipliers from the c matrix.

The H item is negative—an apparently impossible result which is, however, meaningless in view of its large standard error. Indeed, even the value of D is less than its standard error, a result which is to be expected in view of the overwhelming preponderance of non-heritable as opposed to heritable variation. This low value of D would be expected to make selection for increase or decrease of chaeta number largely ineffective. As we shall see, however, in Section 17, the heritable variance was not really absent. It was only hidden in the genotypes. Its release made selection effective in the third and later generations (Mather, 1941).

14. BACKCROSSES

On backcrossing an F_1 to either of its true breeding parents, each gene will be homozygous in half the progeny, and heterozygous in the other half. The contribution of gene **A**-**a** to the mean magnitude of the two backcrosses, as measured from the mid-parent, will therefore be

$$B_1 \quad \tfrac{1}{2}(d_a+h_a), \qquad B_2 \quad \tfrac{1}{2}(h_a-d_a)$$

B_1 being the backcross to the **AA** parent homozygous for the allelomorph giving the + deviation, and B_2 that to the **aa** parent.

The contributions of **A**-**a** to the variances of the backcrosses can easily be shown to be

$$B_1 \quad \tfrac{1}{4}(d_a-h_a)^2, \qquad B_2 \quad \tfrac{1}{4}(d_a+h_a)^2$$

The contributions of d and h to these variances are not separable

as they stand, but if the variances are summed, **A-a** contributes $\frac{1}{2}(d_a^2+h_a^2)$ to the total, and the contributions of d and h can now be separated.

Then, with independent genes, the summed heritable variances of the backcross ($V_{B1}+V_{B2}$) will be $\frac{1}{2}S(d_a^2)+\frac{1}{2}S(h_a^2)$ or $\frac{1}{2}D+\frac{1}{2}H$. This compares with $\frac{1}{2}D+\frac{1}{4}H$ for V_{F2}, and hence the excess of $V_{B1}+V_{B2}$ over V_{F2} must be a measure of $\frac{1}{4}H$. It will indicate the presence of dominance, and this irrespective of the direction of dominance for each gene, because $H=S(h^2)$.

The difference between the variances of the two backcrosses is $S(dh)$, the sign of h here being taken into account. This difference must be zero wherever either the parents are of equal magnitude or the F_1 equals the mid-parent; for in these cases the d and h items are balanced respectively in the sense that the sum of the dh products with a positive sign will be equalled by the sum of those with a negative sign. Where, however, $S(dh)$ does not equal zero it supplies additional evidence of the existence of dominance. At the same time it supplements the evidence from the relation of F_1 to mid-parent in showing which parent carries the preponderance of dominant allelomorphs, for, as we have seen, the backcross to this parent has the lower variance.

Powers (1942) has used this method for showing the dominance of the genes governing fruit weight (expressed on a log scale) in the tomato cross Danmark × Red Currant. The parental means were 1·672 and −0·053, giving a mid-parent of 0·809. The F_1 mean was 0·707, suggesting preponderant dominance of the genes from the smaller parent, Red Currant. The heritable portions of the backcross and F_2 variances Powers estimated as B_{RC} 0·01353, B_D 0·01907, F_2 0·03192. The summed backcross variance exceeds that of F_2 by 0·00068 and so gives $H=0{\cdot}00272$. It then follows that $D=0{\cdot}06248$ and $S(dh)=0{\cdot}00554$. Dominance seems clear, though it is not marked. Furthermore, $S(dh)$ indicates that the genes from the small fruiting parent are preponderantly dominant, as has already been suggested by the comparison of F_1 mean with the mid-parent.

Dominance appears to be more marked in the case of corolla shape (measured as 1000 times log tube length + log lobe length) in the species cross *Nicotiana Langsdorfii* × *N. Sanderae* (Smith, 1937). The parental means were $\bar{S}=1292$ and $\bar{L}=37$, giving a mid-parent of 664·5, as compared with an F_1 mean of 742. The average variance of the reciprocal backcrosses to *N. Sanderae* was 85·5, and of that to *N. Langsdorfii* 98·5. $S(dh)$ is thus in the direction

agreeing with the excess of $\bar{F}_1$ over the mid-parent. Each backcross variance includes a non-heritable item which may be estimated, from the average of parental and F_1 variances, as 42. V_{F2} contains a similar item. Deduction of this quantity leaves 43·5 and 56·5 as the heritable components of the two average backcross variances and 88·5 as that of the average F_2 variance. Then $\frac{1}{2}D+\frac{1}{2}H=43{\cdot}5+56{\cdot}5=100$, and $\frac{1}{2}D+\frac{1}{4}H=88{\cdot}5$, so that $H=46$ and $D=154$ (Fig. 7). If we care to assume that h and d are constant in

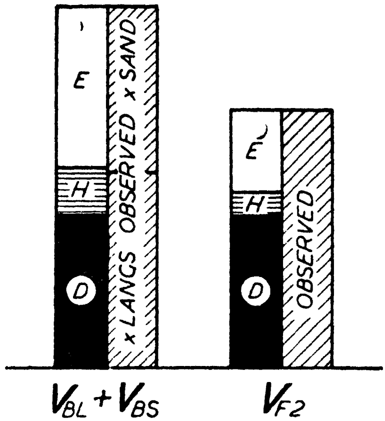


FIG. 7

The values observed for the F_2 and pooled backcross variances of corolla length in *Nicotiana Langsdorfii* × *N Sanderae* (Smith, 1937), and their compositions in terms of D, H and E. The expected totals and observed values agree perfectly, as only these data were available for the separation of D and H

magnitude, though not of necessity in sign, for all the k genes involved, $\sqrt{\frac{H}{D}}=\sqrt{\frac{46}{154}}=0{\cdot}55=\frac{h}{d}$ and provides therefore an estimate of the degree of dominance.

If the individuals of backcross families B_1 and B_2 are again backcrossed to the parents P_1 and P_2, each individual being backcrossed to both parents, further estimates of D and H can be obtained. Let B_{11} be the backcross of B_1 individuals (themselves from the backcross $F_1 \times P_1$) to P_1, B_{12} the backcross of these same individuals to P_2 (i.e. $[F_1 \times P_1] \times P_2$), and so on. The mean variances of the B_{11} ($\bar{V}_{B11}$) and B_{21} ($\bar{V}_{B21}$) progenies are each $\frac{1}{8}S(d-h)^2$ and of B_{12} ($\bar{V}_{B12}$) and B_{22} ($\bar{V}_{B22}$) are $\frac{1}{8}S(d+h)^2$ so that

$$\bar{V}_{B11}+\bar{V}_{B12}=\bar{V}_{B21}+\bar{V}_{B22}=\tfrac{1}{4}D+\tfrac{1}{4}H$$

The variances of the means of B_{11} ($V_{\overline{B11}}$) and B_{21} ($V_{\overline{B21}}$) progenies are each $\frac{1}{16}S(d-h)^2$ and of B_{12} ($V_{\overline{B12}}$) and B_{22} ($V_{\overline{B22}}$) are $\frac{1}{16}S(d+h)^2$ so affording, by appropriate summation, an estimate of $\frac{1}{8}D+\frac{1}{8}H$. All these variances will, of course, also contain non-heritable components.

The covariance of the means of B_{11}, &c., with the values of B_1 or B_2 individuals from which they were derived are even more informative. Covariance B_{11} with B_1 ($W_{B1/B11}$) is $\frac{1}{8}S(d-h)^2$, and B_{22} with B_2 ($W_{B2/B22}$) is $\frac{1}{8}S(d+h)^2$, giving on summation $\frac{1}{4}D+\frac{1}{4}H$. Covariances B_{12} with B_1 ($W_{B1/B12}$), and B_{21} with B_2 ($W_{B2/B21}$), however, are both $\frac{1}{8}S(d^2-h^2)$ or $\frac{1}{8}D-\frac{1}{8}H$, and on summing give $\frac{1}{4}D-\frac{1}{4}H$. D and H may be separately estimated solely by the use of these covariances, which, of course, also have no non-heritable item. Furthermore, the estimates of D and H will be equally precise—a marked advantage for the detection of dominance over the unequal precision of the estimates obtained from F_2 and its derivatives. Unfortunately no double backcross data are available for this method of separation to be tested further.

It is, however, clear that continued backcrossing should form part of any experimental programme aimed at partitioning polygenic variation, unless, of course, it is rendered impossible by technical difficulties.

TABLE 17

Components of Variation in Backcross Progenies

$V_{B1}+V_{B2}$	—Sum of variance of B_1 and B_2	$\frac{1}{2}D+\frac{1}{2}H+2E_1$
$V_{B1}-V_{B2}$	—Difference of variance of B_1 and B_2	$S(dh)$
$\bar{V}_{B11}+\bar{V}_{B12}$ / $\bar{V}_{B21}+\bar{V}_{B22}$	—Sum of mean variance of B_{11} and B_{12} or B_{21} and B_{22}	$\frac{1}{4}D+\frac{1}{4}H+2E_1$
$\bar{V}_{B11}-\bar{V}_{B12}$ / $\bar{V}_{B21}-\bar{V}_{B22}$	—Difference of mean variance of B_{11} and B_{12} or B_{21} and B_{22}	$\frac{1}{2}S(dh)$
$V_{\overline{B11}}+V_{\overline{B12}}$ / $V_{\overline{B21}}+V_{\overline{B22}}$	—Sum of variance of means of B_{11} and B_{12} or B_{21} and B_{22}	$\frac{1}{8}D+\frac{1}{8}H+2E_2$
$V_{\overline{B11}}-V_{\overline{B12}}$ / $V_{\overline{B21}}-V_{\overline{B22}}$	—Difference of variance of means of B_{11} and B_{12} or B_{21} and B_{22}	$\frac{1}{4}S(dh)$
$W_{B1/B11}+W_{B2/B22}$	—Sum of covariance of B_1/B_{11} and B_2/B_{22}	$\frac{1}{4}D+\frac{1}{4}H$
$W_{B1/B11}-W_{B2/B22}$	—Difference of covariance of B_1/B_{11} and B_2/B_{22}	$\frac{1}{2}S(dh)$
$W_{B1/B12}$ and $W_{B2/B21}$	Covariances B_1/B_{12} and B_2/B_{21}	$\frac{1}{8}D-\frac{1}{8}H$

***D* and *H* as in Table 9**

15. RANDOMLY BREEDING POPULATIONS

Where the initial cross is between true breeding lines, and where self-mating, intercrossing and backcrossing can be practised at will, a great multiplicity of statistics are available for the estimation of D, H and E. Most organisms will, however, by their own special properties set limits to the range of statistics which can be obtained. In such plants as wheat, oats and barley, self-pollination is easily secured, whereas crossing of any kind is tedious. Data by means of which the contributions to the variation can be separated will therefore generally come from measurement of F_2 and F_3 families, as in the case of Quisenberry's oats. With self-incompatible and dioecious plants, and with most animals, self-mating is precluded. The statistics available will then be those from successive backcross generations and from F_2 and biparental progenies. Either can lead to a satisfactory analysis.

The value of true-breeding parental lines to experiments of this kind is, of course, very great. Using such lines, simple Mendelian theory can be applied to the transmission of the genes, even though they cannot be followed individually in transmission. In particular, we can be sure that, barring selective elimination of certain classes of gametes or zygotes, corresponding homozygotes, i.e. **AA** and **aa**, will be present in equal numbers in the material. This equality is so important that, as we have seen, backcross data can be used to full advantage only when variances and covariances complementary in their contents of homozygotes are summed. A further advantage following from the use of true breeding lines is their availability for the direct assessment of E, and for any backcrossing that it may be desired to undertake. Even where the backcrosses are not intended to play a major part in partitioning the components of variation, their means afford valuable tests of adequacy of scale when compared with parental and F_1 means.

The partition of variation into its components is therefore much easier where true breeding lines are used in the initial cross. It is, however, possible to make a partition even where such lines are not available. If the genetic differences between individuals within two stocks are small compared with those between the stocks, the stocks may be treated as true breeding without serious error. The genetic variation within them is confounded with the non-heritable variation, and this may indeed be of very small consequence in crosses such as those between species.

Where a species is capable both of easy self-fertilization and

of easy crossing, every member of any population may be itself treated as an F_1 between hypothetical parents. Self-pollination will yield an F_2, which may be used in its turn to give both F_3's and biparental progenies. D, H and E may be estimated from such families even in the absence of the hypothetical parents or of a sufficiently large F_1 from which a direct estimate of E could be obtained. With a clonally propagatable plant, this absence of parents would not be felt, since a clone of any individual of any generation could be used to give the necessary estimates of non-heritable variation. There would remain the question of scaling, unless this had been settled from ancillary experiments. A scale could be chosen to make the fall from F_2 mean to mean of all F_3's twice that from mean of all F_3's to mean of all F_4's, though the test of adequacy so obtained would not be sensitive. By these means the genetical properties of the various individuals of any population could be determined by treating each individual as a unique F_1. When compared with each other these individuals would give a picture of the genetical properties and composition of the population as a whole.

The success of this method of analysing a population of unknown composition and of unknown gene frequencies depends on enforcing a situation, by the initial selfing of the individual, where the gene frequencies become known and gene behaviour predictable from Mendelian principles. The same type of analysis can be put to good use in at least two ways in the later stages of an experiment which itself began with a cross between true breeding lines. First, if each F_2 plant is regarded as an F_1, its F_3 as the corresponding F_2, and so on, the F_2 population can be analysed plant by plant and its composition compared with that expected on the basis of the first analysis which will have already been obtained in the experiment and in which the F_2 was used as a whole for the understanding of the properties of F_1. Secondly, lines raised from F_2 by selective breeding can be analysed in this way in later generations in order to observe the effects of the selection on the decay of variability. In both cases, the parental lines can be employed to give direct estimates of E_1 and the question of scale will already have been settled.

Still other means must be employed where it is desired to undertake the analysis of a population of unknown constitution, whose constituent individuals cannot be self-fertilized. Scaling problems can only be settled by ancillary experiments or considerations, unless it is proposed to undertake the whole analysis by first pro-

ducing a number of inbred lines, intercrossing these and then proceeding as before—a task which will usually be prohibitive if only by its requirements in time. Where a single true breeding line is available, the heterogenic population may be analysed by successive backcrossing of single individuals to it. Otherwise the population must be treated as it stands.

The early biometricians developed the method of correlations between relatives for this purpose of analysing populations over whose breeding no control could be exercised. This method has been thoroughly reviewed from the standpoint of Mendelian inheritance by Fisher (1918), but it is not confined in its application to the analysis of heritable variation. It has been extensively used for the partitioning of non-heritable variation into its various components (see Wright, 1934*a*; Lush, 1943). Its use depends on the fact that where a correlation r exists between two variates, a proportion r^2 of the variance of one variate may be accounted for by reference to variation in the other, leaving $1-r^2$ as residual variation for which other causes must be sought. In this way, an analysis of variance may be arrived at.

When used for the separation and analysis of heritable variation the method of correlation between relatives is, as would be expected, related to the methods already developed for the special case of crosses between true-breeding stocks. It leads, in fact, to generalized formulae for D and H, and at the same time shows us the special advantages of the type of data already discussed. We shall assume that the scale on which measurements are taken is adequate for the elimination of interaction. If it is not, a special term must be introduced into the analysis to take care of the interaction (Fisher, 1918, Wright, 1935).

Where, in a population mating at random, the two allelomorphs **A** and **a** of a gene occur with frequencies u_a and v_a $(=1-u_a)$, respectively, in the gametes taken as a whole, the three genotypes **AA**, **Aa**, and **aa** will occur with the average frequencies $u_a^2 : 2u_a v_a : v_a^2$ in each generation. Then, if as before the contributions made by the three genotypes to the character metric, x, are d_a, h_a and $-d_a$ respectively, the mean will be $d_a(u_a-v_a)+2h_a u_a v_a$. The contribution which this gene makes to the variance of the population will be

$$d_a^2(u_a^2+v_a^2)+h_a^2 2u_a v_a-[d_a(u_a-v_a)+2h_a u_a v_a]^2$$

the last term being the correction for the mean. On simplification this expression becomes

$$2u_a v_a[d_a^2+2h_a d_a(v_a-u_a)+h_a^2(1-2u_a v_a)]$$

This can be recast in the form

$$2u_a v_a[d_a^2+2h_a d_a(v_a-u_a)+h_a^2(v_a-u_a)^2+2h_a^2u_a v_a]$$

or

$$2u_a v_a\{[d_a+h_a(v_a-u_a)]^2+2h_a^2u_a v_a\}$$

Where the genes are independent in action and unlinked in transmission, the total heritable variation will be the sum of the series of such items, one from each gene, viz.

$$S\{2uv[d+h(v-u)]^2+4h^2u^2v^2\}$$

It will then be seen that if we put

$$D=S\{4uv[d+h(v-u)]^2\} \qquad \text{and} \qquad H=S(16h^2u^2v^2)$$

the heritable variance becomes $\frac{1}{2}D+\frac{1}{4}H$. Apart from sampling variation, this heritable variance will be constant from generation to generation.

When $u=v=\frac{1}{2}$ for all genes, as in the F_2 of a cross between two true breeding lines, these general expressions for D and H reduce

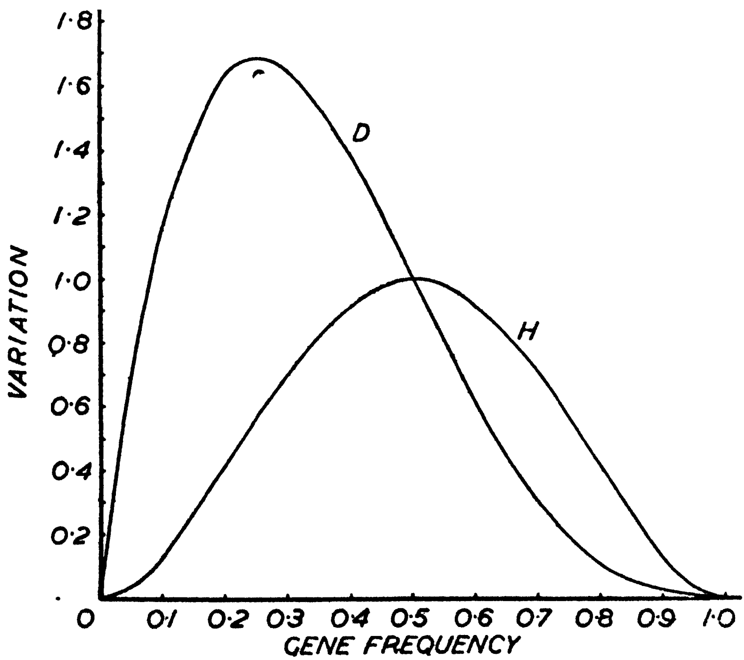


FIG. 8

Change in the contributions made by a gene to D and H according to the frequency of its dominant allelomorph in a randomly breeding population In calculating the variation it was assumed that $d=h=1$

to $S(d^2)$ and $S(h^2)$, and the heritable variance itself reduces, as it should, to that already found for V_{F_2}. Thus if, and only if $u=v=\frac{1}{2}$ the contribution made by d and h to the heritable variation may be separated in the analysis. Otherwise D includes some effect of h, and H is correspondingly less than the summed effects of all the squared h deviations. D may be greater or less than $S(d^2)$, according to the relative frequencies of the more dominant and more recessive allelomorphs (Figs. 8 and 9).

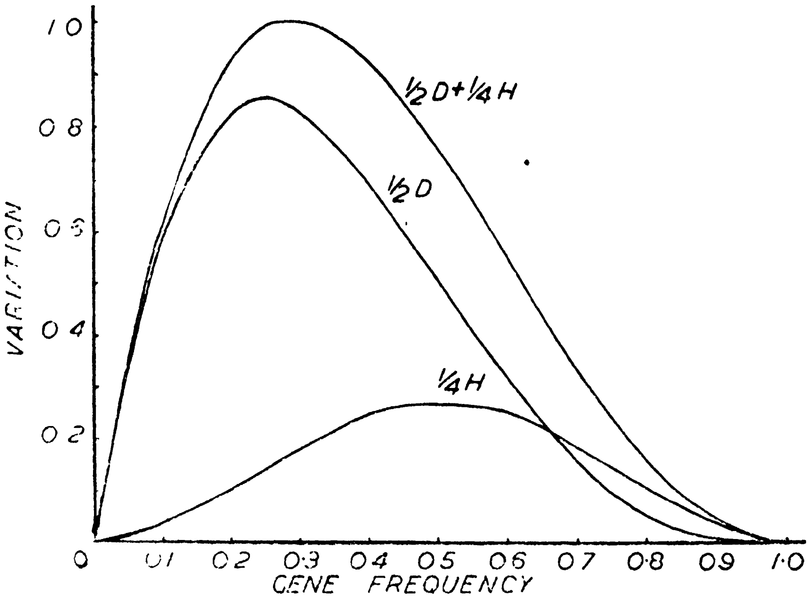


FIG. 9

Change in the contribution $(\frac{1}{2}D+\frac{1}{4}H)$ made by a gene to the variation of a randomly breeding population according to the frequency of its dominant allelomorph. The separate items $\frac{1}{2}D$ and $\frac{1}{4}H$ are also shown. Calculations are based on $d=h=1$

Turning next to the parent-offspring covariance, the parents will be of the three types **AA**, **Aa**, **aa** with the relative frequencies $u_a^2 : 2u_a v_a : v_a^2$. The population of gametes, with which those of each parent will combine in giving the next generation, will carry **A** and **a** with the relative frequencies u_a and v_a. The offspring of **AA** parents will therefore be **AA** in u_a of cases and **Aa** in v_a of cases. Now the former group will have an expression, in regard to this gene, of d_a and the latter an expression of h_a. Taking all the types of parent in turn we can in this way build up a table

showing the frequencies of the various relations of expression in parent and offspring thus:

				Offspring		
				AA	Aa	aa
				d_a	h_a	$-d_a$
	u_a^2	AA	d_a	u_a^3	$u_a^2 v_a$	—
Parents	$2u_a v_a$	Aa	h_a	$u_a^2 v_a$	$u_a v_a$	$u_a v_a^2$
	v_a^2	aa	$-d_a$	—	$u_a v_a^2$	v_a^3

The sum of cross-products of parent and offspring will therefore be

$$d_a^2(u_a^3+v_a^3)+2d_a h_a(u_a^2 v_a-u_a v_a^2)+h_a^2 u_a v_a$$

from which must be deducted a term correcting for the means of parents and offspring. This will be the same as that used in calculating the variance, since the mean measurement of all offspring is the same as that of all parents.

The contribution of this gene to the covariance of parent and offspring thus becomes

$$d_a^2[u_a^3+v_a^3-(u_a-v_a)^2]+2h_a d_a[u_a^2 v_a-u_a v_a^2-2u_a v_a(u_a-v_a)]+h_a^2(u_a v_a-4u_a^2 v_a^2)$$

which reduces to

$$u_a v_a d_a^2+2u_a v_a(v_a-u_a)h_a d_a+u_a v_a(1-4u_a+4u_a^2)h_a^2$$

or
$$u_a v_a[d_a+h(v_a-u_a)]^2$$

and summing over all independent genes, we find $W_{P/O}=\frac{1}{2}D$, since a covariance has no non-heritable component.

The covariance of full sibs may be calculated similarly. There is but one complication to bear in mind. In a family from, say, the cross **AA** × **Aa**, equal numbers of **AA** and **Aa** offspring are expected. Then of pairs of sibs taken at random, $\frac{1}{4}$ will be both **AA**, $\frac{1}{4}$ will be both **Aa** and $\frac{1}{2}$ will include a sib of each kind. Such a cross between **AA** and **Aa** parents will be expected with frequency $u_a^2 . 2u_a v_a$, and so will contribute $\frac{1}{4}2u_a^3 v_a d_a^2+\frac{1}{4}.2u_a^3 v_a h_a^2+\frac{1}{2}u_a^3 v_a d_a h_a$ to the sum of cross-products. The correction term will be as before and so we obtain for the contribution of **A**-**a** to the covariance of full sibs

$$u_a v_a[d_a^2+2h_a d_a(v_a-u_a)+h_a^2(1-3u_a v_a)]$$

or
$$u_a v_a\{[d_a+h_a(v_a-u_a)]^2+h_a^2 u_a v_a\}$$

which on summation of all independent genes yields

$$W_{S/S}=\frac{1}{4}D+\frac{1}{16}H$$

The covariance of half-sibs is similarly $\frac{1}{8}D$. Those for other relatives can be calculated in the same way.

The correlation between parent and offspring is found as

$$r_{P/O} = \frac{W_{P/O}}{\sqrt{V_P . V_O}} = \frac{W_{P/O}}{V}, \text{ since } V_P = V_O = V$$

$$= \frac{\frac{1}{4}D}{\frac{1}{2}D + \frac{1}{4}H + E}$$

The correlation between full sibs is similarly

$$r_{S/S} = \frac{W_{S/S}}{V} = \frac{\frac{1}{4}D + \frac{1}{16}H}{\frac{1}{2}D + \frac{1}{4}H + E}$$

Knowledge of these two correlations of itself enables us therefore to estimate the relative magnitudes of D, H and E.

Fisher (1918) records that Pearson and Lee's data yield $r_{P/O} = 0{\cdot}4180$ and $r_{S/S} = 0{\cdot}4619$ for the human cubit measurement. Now

$$\tfrac{1}{4}D + \tfrac{1}{16}H = r_{S/S}(\tfrac{1}{2}D + \tfrac{1}{4}H + E)$$

and

$$\tfrac{1}{4}D = r_{P/O}(\tfrac{1}{2}D + \tfrac{1}{4}H + E)$$

The general solution to these equations is

$$\frac{H}{D} = \frac{4(r_{S/S} - r_{P/O})}{r_{P/O}} \quad \text{and} \quad \frac{E}{D} = \frac{1 - 2r_{P/O} - \frac{H}{D}r_{P/O}}{4r_{P/O}}$$

Substituting we find $H = 0{\cdot}4201D$ and $E = -0{\cdot}0069D$.

In other words, the non-heritable variation must be very small, our estimate becoming negative through sampling error, while H is nearly half as large as D. Taking E as zero, the variation in the population would be partitionable into 17% due solely to dominance and 83% also including a portion due to dominance but mainly reflecting the d items of variation.

These results can, however, only be regarded as approximations, because the analysis itself is over simple for two reasons. In the first place, the assumption of random mating is not fully justified in man. There is, in fact, a marital correlation of 0·1 between the cubit measurements of mates. This must result in higher values for both $r_{P/O}$ and $r_{S/S}$ than would be obtained with the same genetic structure under a system of random mating. Our estimate of E will therefore be too low, and H seems also to be underestimated. The results are not, however, made seriously inaccurate by the assumption of random sampling. Fisher describes a method of making allowance for the marital correlation. On using it he

still finds that the contribution made by E to the variation is negligible, but that 17% of the variation is due to homogany and that the ratio of the H and D contributions is approximately 1 : 3. The values reached by both methods of partition are, however, subject to considerable sampling errors (Fisher, 1918).

The second difficulty arises from the assumption that the non-heritable variation is simply additive to the heritable. This cannot in fact be the case in man. As a result of family life both parents and offspring on the one hand and sibs on the other will tend to enjoy environmental conditions more alike than the environments of unrelated persons. Genetical similarity is, therefore, accompanied by some similarity of environment. The estimate of E obtained in the analysis must tend to be spuriously low for this reason, as well as by reason of the marital correlation. It is, however, difficult to believe that if non-heritable variation between families were at all sizeable, the E component would be found to be negligible in the analysis. Though our estimate must be too small, E is not likely to constitute more than a small fraction of the variation between the individuals measured even when estimated without bias. Furthermore, the estimated ratio of H to D should not be seriously affected by this false assumption about the distribution of environmental differences.

These difficulties with the environment and the mating system are likely to arise whenever the data are obtained, as they are with man, by simple observation. Where experiment is possible bias due to difficulties of these kinds may be avoided, or at least materially reduced. One experimental design which avoids at least those difficulties which arise from a marital correlation, is that known as diallel crossing. In such an experiment each of the male parents is mated in turn to each of the female parents, so that a progeny is raised from every possible cross. The genetic values of the various males on the one hand, and of the various females on the other, can therefore be compared with some precision, because all of them are assayed over the same range of mates.

The frequencies of the various types of mating, the mean measurement and the variance of the offspring from each mating are given in respect of gene **A**-**a** in Table 18, assuming the various parental genotypes to be used with frequencies proportional to those found in the randomly breeding population. The mean taken over all families, assumed to be of equal size, is of course that of the original population. The mean of all offspring from one parent varies with the parent itself, the covariance being $\frac{1}{4}D$, as we have

already seen. The regression of offspring on parent is equal to $r_{P/O}$, and therefore has the maximum value of $\frac{1}{2}$ when $H=E=0$.

TABLE 18
Diallel Crosses
Mean Expressions and Variances in Progenies

Parents	Male AA u_a^2	Male Aa $2u_av_a$	Male aa v_a^2	Mean
AA u_a^2	$\bar{x}=d_a$ $V_x=0$	$\frac{1}{2}(d_a+h_a)$ $\frac{1}{4}(d_a-h_a)^2$	h_a 0	$u_ad_a+v_ah_a$
Female Aa $2u_av_a$	$\frac{1}{2}(d_a+h_a)$ $\frac{1}{4}(d_a-h_a)^2$	$\frac{1}{2}h_a$ $\frac{1}{4}(2d_a^2+h)_a^2$	$\frac{1}{2}(h_a-d_a)$ $\frac{1}{4}(d_a+h_a)^2$	$\frac{1}{2}[d_a(u_a-v_a)+h_a]$
aa $2v_a^2$	h_a 0	$\frac{1}{2}(h_a-d_a)$ $\frac{1}{4}(d_a+h_a)^2$	$-d_a$ 0	u_ah-v_ad
Mean	$u_ad_a+v_ah_a$	$\frac{1}{2}[d_a(u_a-v_a)+h_a]$	$u_ah_a-v_ad_a$	$(u_a-v_a)d_a+2u_av_ah_a$

In addition to the variance and the different covariances which may be found from such data as are available from man, diallel crosses afford a number of new statistics which may be used in the evaluation of D, H and E. These are listed in Table 19. Some of the variances and covariances in this table represent the general cases of the statistics derivable from biparental progenies and maternal progenies of the third generation from crosses between true-breeding lines as given in Table 9. They have the same structure in terms of D, H and E; but where originating from the cross between true breeding lines, $u=v=\frac{1}{2}$ for all genes, and so D and H are themselves defined more closely.

This range of statistics obtainable from a diallel crossing experiment is sufficient to yield an adequate partition of the variance into its components, provided that sufficient parents are used. The chief difficulty will generally arise from the geometric increase in the number of progenies required to complete the experiment when the number of parents increases arithmetically. There is, however, no necessity for the numbers of parents of the two sexes to be kept equal. In general, it will be easier to test a limited number of male parents on a wider range of female parents. The structures of the various statistics will still be the same, whether

TABLE 19

Components of Variance and Co-Variance in Randomly Breeding Populations

$D = S\{4uv[d+h(v-u)]^2\}$ $\qquad$ $H = S(16h^2u^2v^2)$

E_1 = non-heritable variance of individuals $\qquad$ E_2 = non-heritable variance of means

General		
(V_{F2})	Variance of individuals	$=\frac{1}{2}D+\frac{1}{4}H+E_1$
$(W_{F2/B1P})$	Covariance Parent/Offspring	$=\frac{1}{4}D$
	Covariance of full sibs	$=\frac{1}{4}D+\frac{1}{16}H$
	Covariance of half sibs	$=\frac{1}{8}D$
In Diallel Crosses		
$(V_{\overline{B1P}})$	Variance of all family means	$=\frac{1}{4}D+\frac{1}{16}H+E_2$
$(V_{\overline{MAT}})$	Variance of means of all offspring of single parents	$=\frac{1}{8}D+E_2$ (or E_3 under special circumstances)
$(\bar{V}_{B1P})$	Mean variance of all families	$=\frac{1}{4}D+\frac{3}{16}H+E_1$
	Mean variance of family means from single parents	$=\frac{1}{8}D+\frac{1}{16}H+E_2$
$(\bar{V}_{MAT})$	Mean variance of all offspring from single parents	$=\frac{3}{8}D+\frac{1}{4}H+E_1$

A number of these statistics are general forms of some listed in Table 9 obtained from the descendants of a cross between true-breeding lines. These comparisons are indicated above by the symbols in brackets.

obtained by dividing the families up upon the basis of the male parents or of the females; but their precisions will not, of course, be identical when the numbers of male and female parents differ.

A similar experimental design is used in progeny testing bulls. A limited range of males is tested over a wider range of females, but the same females are not of necessity used for testing all the males in turn. Provided that the set of females used for mating to each male is itself a fair sample from the population of females, no bias is introduced. An additional source of sampling error is, however, obviously brought in. The necessity is thereby made the greater for securing a range of statistics sufficiently wide to provide empirical estimates of the error of estimation of *D*, *H* and *E*, in the way illustrated earlier in this chapter. Indeed, it is clearly a general principle of experiments made in order to partition the variation of a population or the segregating generations of a cross, that their designs should yield not merely the statistics necessary for the partition, but also the means of determining the errors of estimations of the components of variation which are obtained by the partition. The more statistics there are made available, the greater will be our knowledge of the errors of estimation, and the further will it be possible to pursue the analysis.

CHAPTER 5

LINKAGE

16. THE EFFECT OF LINKAGE

THE CONTRIBUTIONS of the various genes to the fixable and unfixable components of the genetic variation are additive, in the way assumed in the previous chapter, only if the genes themselves are independent both in action and inheritance. Independence in action means, of course, that the increment added to the character in question by one gene is uninfluenced by the remainder of the genotype. As we have already seen, this must usually be a problem of scaling, and it has already been discussed in that connection. No further general comment is required, though the effects of certain special types of genic interaction on linkage detection will be considered later.

Independence of two genes in inheritance means that the two allelomorphs of one gene are equally likely to be transmitted from parent to offspring together with a given allelomorph of the second gene. Apart from special cases, therefore, dependence of genes in inheritance means linkage.

Where the genes in question have no lethal or semi-lethal effects, linkage does not effect the frequencies with which the allelomorphs of each gene are recovered in segregating generations. It only leads to particular combinations of these alternatives appearing with frequencies other than those expected from independence. On a scale where the increments added to the phenotype by the various genes are themselves additive, the total effect of a gene on the phenotypes of a family of given size will be the same, apart from sampling variation, no matter what its linkage relations may be. The relative frequencies of the particular combinations in which the allelomorphs occur with other genes will have no effect, because every one which is over-common will be balanced by another being correspondingly rare. Linkage, therefore, can of itself have no effect on the mean measurements of segregating families, provided that the scale chosen for representing the phenotypes satisfies the scaling tests developed in Chapter 3. Equally then the operation of linkage does not vitiate these scaling tests.

Linkage, though not affecting the means, shows its effect in the second degree statistics, the variances and covariances used in

partitioning the variation. Consider the simplest case of two genes **A-a** and **B-b**. When in the coupling phase with recombination value $p(=1-q)$, the ten genotypes of F_2 are expected with the frequencies shown in Table 20. This table also gives the phenotypic deviations of each class from the mid-parent, and the mean phenotypes of the corresponding F_3 families. It is not difficult to see from these data that the mean phenotype of F_2 is unaffected by

TABLE 20

Frequencies, F_2 Phenotypes and Mean F_3 Phenotypes of the 10 Genotypic Classes in an F_2 for Two Coupled Genes

The arrangement within each cell is :—
Frequency
F_2 phenotype
Mean F_3 phenotype

	AA	Aa	aa
BB	q^2 d_a+d_b d_a+d_b	$2pq$ h_a+d_b $\frac{1}{2}h_a+d_b$	p^2 $-d_a+d_b$ $-d_a+d_b$
Bb	$2pq$ d_a+h_b $d_a+\frac{1}{2}h_b$	C $2q^2$ h_a+h_b $\frac{1}{2}(h_a+h_b)$ $2p^2$ h_a+h_b $\frac{1}{2}(h_a+h_b)$ R	$2pq$ $-d_a+h_b$ $-d_a+\frac{1}{2}h_b$
bb	p^2 d_a-d_b d_a-d_b	$2pq$ h_a-d_b $\frac{1}{2}h_a-d_b$	q^2 $-d_a-d_b$ $-d_a-d_b$

All frequencies should be divided by 4

the linkage, being $\frac{1}{2}(h_a+h_b)$ as before. But the sum of squares of deviations from the mid-parent is now

$$\tfrac{1}{4}[q^2(d_a+d_b)^2+2pq(d_a+h_b)^2 \;\ldots\; +q^2(-d_a-d_b)^2]$$

On subtracting $[\frac{1}{2}(h_a+h_b)]^2$ to correct for the departure of the F_2 mean from the mid-parent, the sum of squares of deviations from the mean, and with it the heritable variance (since the frequencies sum to unity), becomes

$$V_{F2}=\tfrac{1}{2}[d_a^2+d_b^2+2(1-2p)d_ad_b]+\tfrac{1}{4}[h_a^2+h_b^2+2(1-2p)^2h_ah_b].$$

Two new terms are to be observed in this expression, both

involving the recombination value, in one case combined with the product $d_a d_b$ and in the other with $h_a h_b$. With free recombination $p=\frac{1}{2}$, so that $1-2p=0$ and these new terms vanish to leave the expressions obtained in the last chapter. When linkage is complete $p=0$, giving $1-2p=1$ and aside from non-heritable variation $V_{F2}=\frac{1}{2}(d_a+d_b)^2+\frac{1}{4}(h_a+h_b)^2$. The two genes then act as one. Even, however, where recombination occurs, recombinant types will be rare if p is small, and the genes will effectively act as one except in so far as selection may isolate one of the rare recombinants.

If we now write $\quad D=d_a^2+d_b^2+2(1-2p)d_a d_b$

and $\quad H=h_a^2+h_b^2+2(1-2p)^2 h_a h_b$

in place of the earlier definitions of these two components of heritable variation, we can again put $V_{F2}=\frac{1}{2}D+\frac{1}{4}H+E$. It can then be shown that, as before, $V_{\overline{F3}}=\frac{1}{2}D+\frac{1}{16}H+E$ and $W_{F2/F3}=\frac{1}{2}D+\frac{1}{8}H$.

If the two genes are in repulsion, the expression for D is changed to $d_a^2+d_b^2-2(1-2p)d_a d_b$, but, as might be expected, that for H is not changed. It should be noted, however, that the term $2(1-2p)^2 h_a h_b$ will be positive only if h_a and h_b are *reinforcing* one another by acting in the same direction. If they are *opposing* each other, this term must be negative. Thus opposition of the heterozygous increments resembles repulsion of the genes, and reinforcement resembles coupling in their effects on the variances and covariances (Fig. 10). It must nevertheless be remembered that opposition *v.* reinforcement is a physiological distinction, while repulsion *v.* coupling is a mechanical one.

When we turn to the mean variance of F_3 we find that it too can still be written in its old forms of $\bar{V}_{F3}=\frac{1}{4}D+\frac{1}{8}H+E$, but not only are D and H in this expression different from the corresponding items in the case of independent inheritance, they also differ from those given by V_{F2}, &c., with linked inheritance. Taking the coupling use, the four corner genotypes of Table 20 are true breeding, and so give F_3 variances of 0. Two of the remainder will give segregation for **A-a** in F_3 and two more segregation for **B-b**. The doubly heterozygous types in coupling and repulsion will give F_3's having variances like those of F_2 families showing the corresponding phase of linkage. Combining these variances in proportion to their expected frequencies we find, in respect of these two genes,

$$\bar{V}_{F3}=\tfrac{1}{4}[d_a^2+d_b^2+2(1-2p)^2 d_a d_b]+\tfrac{1}{8}[h_a^2+h_b^2+2(1-2p)^2(1-2p+2p^2)h_a h_b]$$

The repulsion case gives the same expression, but with the signs of the term in $d_a d_b$ reversed.

Then $$D = d_a^2 + d_b^2 \pm 2(1-2p)^2 d_a d_b$$
and $$H = h_a^2 + h_b^2 + 2(1-2p)^2(1-2p+2p^2) h_a h_b$$
the $\pm$ indicating the change in sign of the $d_a d_b$ term according to the phase of linkage. These expressions for D and H differ from those given by V_{F2}, V_{F3} and $W_{F2/F3}$ in that $(1-2p)^2$ replaces $(1-2p)$

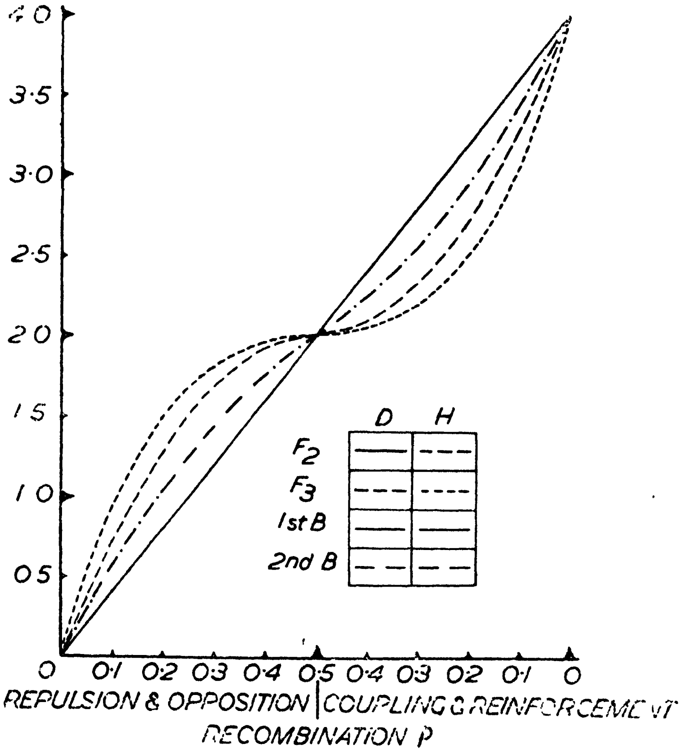


FIG. 10

Change in the contribution made by two segregating genes to D and H in F_2, F_3 and backcrosses according to the genes' linkage relations Calculations are based on $d_a = d_b = h_a = h_b = 1$

and $(1-2p)^2(1-2p+2p^2)$ replaces $(1-2p)^2$ in the $d_a d_b$ and $h_a h_b$ terms respectively.

In the same way $V_{\overline{BIP}}$ and $W_{F2\cdot BIP}$ can still be written as $\frac{1}{4}D + \frac{1}{16}H + E$ and $\frac{1}{4}D$ respectively, but D and H now have values as in V_{F2}. When we come to the mean variance of biparental progenies we find that $\bar{V}_{BIP} = \frac{1}{4}D + \frac{3}{16}H + E$, but D and H have changed from their V_{F2} values just as they did in the case of $\bar{V}_{F3}$.

Turning to progenies raised by backcrossing, the expectations

for which are given in Table 21, we find that $D=d_a^2+d_b^2+2(1-2p)d_ad_b$ for coupling, and $D=d_a^2+d_b^2-2(1-2p)d_ad_b$ for repulsion, H being given by $h_a^2+h_b^2+2(1-2p)h_ah_b$ irrespective of linkage phase. In H the sign of the term in p will, of course, vary according to the opposition or reinforcement of the h_a and h_b increments. When D and H are redefined in this way the summed variances of the two first backcrosses still equal $\frac{1}{2}D+\frac{1}{2}H+2E$, and the various combinations of variances of means of second backcrosses as well as covariances

TABLE 21

Linkage in Backcrosses. Frequencies and Phenotypes of the Classes

Coupling

×AABB

	AA	Aa
BB	q d_a+d_b	p h_a+d_b
Bb	p d_a+h_b	q h_a+h_b

×aabb

	Aa	aa
Bb	q h_a+h_b	p $-d_a+h_b$
bb	p h_a-d_b	q $-d_a-d_b$

Repulsion

×AAbb

	AA	Aa
Bb	p d_a+h_b	q h_a+h_b
bb	q d_a-d_b	p h_a-d_b

×aaBB

	Aa	aa
BB	p h_a+d_b	q $-d_a+d_b$
Bb	q h_a+h_b	p $-d_a+h_b$

All frequencies should be divided by 2

of means of second backcrosses with their first backcross parents also have the compositions shown in Table 17. Once again, however, the expressions for D and H change when we turn to the mean variances of the second backcross progenies. Though the formulae of Table 17 apply, D is now $d_a^2+d_b^2\pm2(1-2p)(1-p)d_ad_b$ and H is $h_a^2+h_b^2+2(1-2p)(1-p)h_ah_b$, the phase of linkage determining the sign of the recombination term in the expression for D.

Fig. 10 shows the changes in value of D and H from F_2, F_3 and the first (1B) and second (2B) backcrosses with the recombination

fraction, when $d_a=d_b=h_a=h_b=1$. The effects of repulsion and coupling on D are paralleled by those of opposition and reinforcement on H. All the various D's and H's are equal when $p=0$ or $p=0{\cdot}5$, i.e. for complete linkage or free assortment. At any other value of p, the values of D and H in F_3, and in 2B, approach more nearly to those given by free assortment than do the values of D and H in F_2 and 1B. The difference is greatest between $p=0{\cdot}20$ and $p=0{\cdot}30$.

In the absence of linkage, i.e. where $p=0{\cdot}5$, the heritable portion

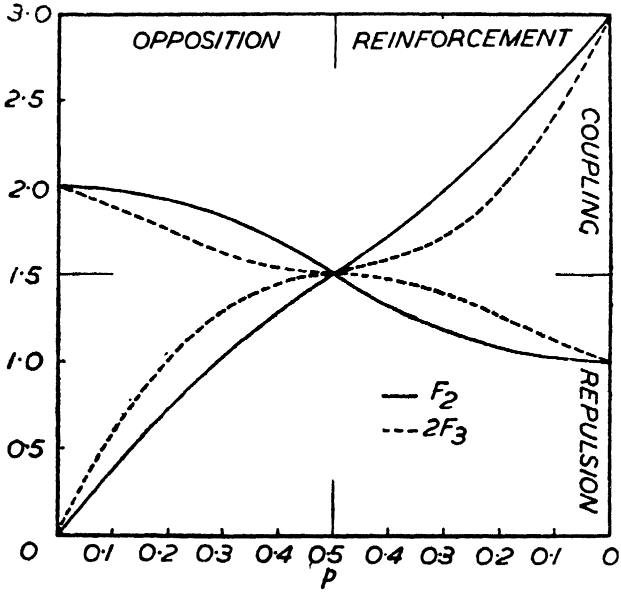


FIG 11

Change in the contribution ($\frac{1}{2}D+\frac{1}{4}H$) made to V_{F2} and $2V_{F3}$ by two segregating genes according to the genes' linkage relations Calculations are based on $d_a=d_b=h_a=h_b=1$

of $\bar{V}_{F3}$ is half that of V_{F2}, and the heritable portion of $\bar{V}_{B11}+\bar{V}_{B12}$ or $\bar{V}_{B21}+\bar{V}_{B22}$ is half that of $V_{B1}+V_{B2}$ (Table 17). $2\bar{V}_{F3}$ and V_{F2} are compared in Fig. 11 over all recombination values in the various combinations of repulsion and coupling with opposition and reinforcement when $d_a=d_b=h_a=h_b=1$. Fig. 12 shows the same comparison for $V_{B1}+V_{B2}$ and $2(\bar{V}_{B11}+\bar{V}_{B12})$ or $2(\bar{V}_{B21}+\bar{V}_{B22})$. Again it will be seen that the effect of linkage on the variances is greatest at the intermediate values of p, whatever the combination of linkage phase and dominance co-operation.

The presence of linkage may be detected by comparing the

magnitudes of the heritable portions of the variances in F_2 and F_3 or in 1B and 2B. With linkage $2\bar{V}_{F3}$ no longer equals V_{F2}, and $2(\bar{V}_{B11}+\bar{V}_{B12})$ or $2(\bar{V}_{B21}+\bar{V}_{B22})$ no longer equals $V_{B1}+V_{B2}$. Before, however, turning to the application of this test, we must consider the general case of linkage of more than two genes.

Cases where three or more linked genes are involved can be worked out by the same methods as were used above for two

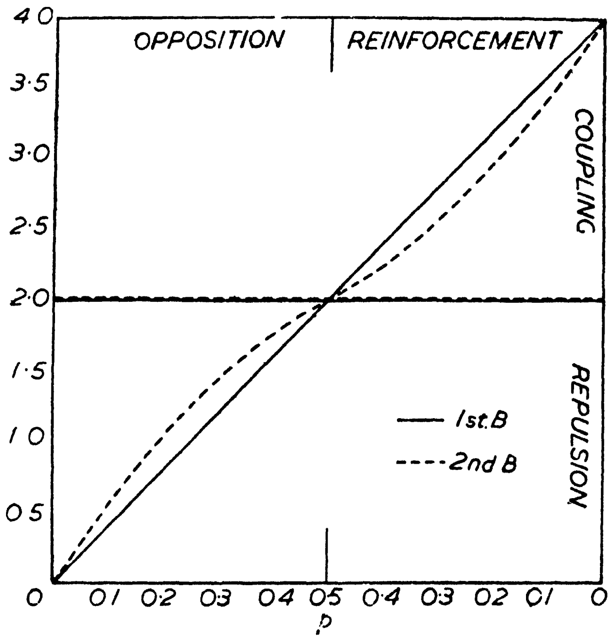


FIG. 12

Change in the contribution $(\frac{1}{2}D+\frac{1}{4}H)$ made by two segregating genes to $V_{B1}+V_{B2}$ and $2(V_{B11}+V_{B22})$ according to the genes' linkage relations Calculations are based on $d_a=d_b=h_a=h_b=1$

genes. The algebra is, however, over-tedious for detailed presentation. In general it appears that the formulae of Tables 9 and 17 still hold, but with changed expressions for D and H. These new expressions are set out in Table 22. S_C and S_R stand for the sums of all the items with the two genes in coupling and repulsion respectively. It should be noted that p is the recombination value shown by the two genes in question irrespective of any gene between them. In the same way the phase of a linkage is independent of intermediate genes. Thus where there are three linked

TABLE 22

General formulae of D and H with Linkage

Generation	D	H
F_2	$S(d^2)+2S_C[(1-2p)d_a d_b]$ $-2S_R[(1-2p)d_a d_b]$	$S(h^2)+2S[(1-2p)^2 h_a h_b]$
F_3	$S(d^2)+2S_C[(1-2p)^2 d_a d_b]$ $-2S_R[(1-2p)^2 d_a d_b]$	$S(h^2)+2S[(1-2p)^2(1-2p+2p^2)h_a h_b]$
1B	$S(d^2)+2S_C[(1-2p)d_a d_b]$ $-2S_R[(1-2p)d_a d_b]$	$S(h^2)+2S[(1-2p)h_a h_b]$
2B	$S(d^2)+2S_C[(1-2p)(1-p)d_a d_b]$ $-2S_R[(1-2p)(1-p)d_a d_b]$	$S(h^2)+2S[(1-2p)(1-p)h_a h_b]$

S_C=sum over all pairs of genes in coupling
S_R=sum over all pairs of genes in repulsion

genes, arranged as $\frac{\mathbf{AbC}}{\mathbf{aBc}}$, the recombination values are **A-B** p_{ab}, **B-C** p_{ac}, and **A-C** p_{ac} ($=p_{ab}+p_{bc}-2cp_{ab}p_{bc}$, where c is the coincidence value). Also **A** and **B** and **B** and **C** are repulsed, but **A** and **C** are coupled. Then in F_2

$$D=d_a^2+d_b^2+d_c^2-2(1-2p_{ab})d_a d_b-2(1-2p_{bc})d_b d_c+2(1-2p_{ac})d_a d_c$$

H is unaffected by linkage phase and so

$$H=h_a^2+h_b^2+h_c^2+2(1-2p_{ab})^2 h_a h_b+2(1-2p_{bc})^2 h_b h_c+2(1-2p_{ac})^2 h_a h_c$$

but the remarks made above about the effect of linkage phase on the value of D will here apply to reinforcement and opposition relations. The F_2 values of D and H apply to $V_{\overline{F3}}$ and $W_{F2/F3}$, i.e. to all cases where only F_2 phenotypes and/or F_3 means are involved. The changed F_3 values are found in $\bar{V}_{F3}$. In the same way the 1B values apply to the variances of the first backcrosses, to the variances of second backcross means, and to the corresponding covariances. The 2B values apply to the mean variances of this generation. The general test for linkage is therefore that of the homogeneity of D and H over the F_2 and F_3, or over 1B and 2B generations. The values of D and H may also vary as a result of sampling error between statistics which are expected to show the same values for them, e.g. between V_{F2} and $V_{\overline{F3}}$. Such differences afford a measure of error variation. Agents, such as genic interaction, can inflate variation of this kind and they must consequently lower the precision of the linkage test. They will not, however, bias the test unless they affect the various statistics differentially. An effect simulating linkage could result only from

the excessive increase or decrease of D and H in $\bar{V}_{F3}$ on the one hand as compared with V_{F2}, $V_{\bar{F3}}$ and $W_{F2/F3}$ taken as a group on the other, or by corresponding differential inflation in the back-crosses. It appears that most forms of genic interaction will not bias the linkage test in this way, though some forms of epistatic action may do so.

The linkage of a number of genes will exert its maximum effect on the variances and covariances when all are coupled and all their heterozygous effects are reinforcing. All the terms containing p will then be positive. The effects of repulsion and opposition can never be so great, except where only two genes are concerned, since more than two genes can be neither all repulsed nor all opposed to one another. The maximum effects of repulsion and opposition might be expected when adjacent genes are in repulsion and opposition. Even in such a case, however, the 1st, 3rd, 5th, &c., must be coupled and reinforcing, as must the 2nd, 4th, 6th, &c. Inequality of the d and h increments of the various genes will also reduce the effect of linkage on the variances and covariances.

The effect of linkage on the value of a statistic could be zero even though linkage were in fact present; for the coupling and repulsion items, as well as the reinforcement and opposition items, could balance. The circumstances in which such a balance will be achieved must depend on the relative magnitudes of effect of the genes and on their recombination frequencies, as well as on their phasic relations. Even, however, where linkage items balance in D and H of F_2 they will not do so exactly in D and H of F_3. The test for linkage would seem to hold good, though under such circumstances it may well be insensitive.

These various considerations will, perhaps, be more easily seen from Fig. 13. This compares the heritable portions of V_{F2} and $2\bar{V}_{F3}$ for the various arrangements of four genes. It is assumed that all the d and h increments are of unit magnitude (though the h increments differ in sign as indicated), and that there is no interference in recombination between different segments, i.e. that all coincidence values are 1. The three primary recombination values p_{ab}, p_{bc} and p_{cd} are further assumed all to be 0·125. Wherever V_{F2} markedly exceeds or falls short of the value it would have in the absence of linkage, $2\bar{V}_{F3}$ occupies an intermediate position. In such cases D and H are changed in the same direction. But where D and H depart from their unlinked expectations in opposite ways, the pooled departure being therefore a relatively small one, $2\bar{V}_{F3}$ may depart more widely than V_{F2} or it may even depart in the opposite

direction. The difference between V_{F2} and $2\bar{V}_{F3}$ must then be following that of the D item, which is itself tending towards the unlinked equilibrium value, since D preponderates over H in determining the magnitudes and changes of these variances.

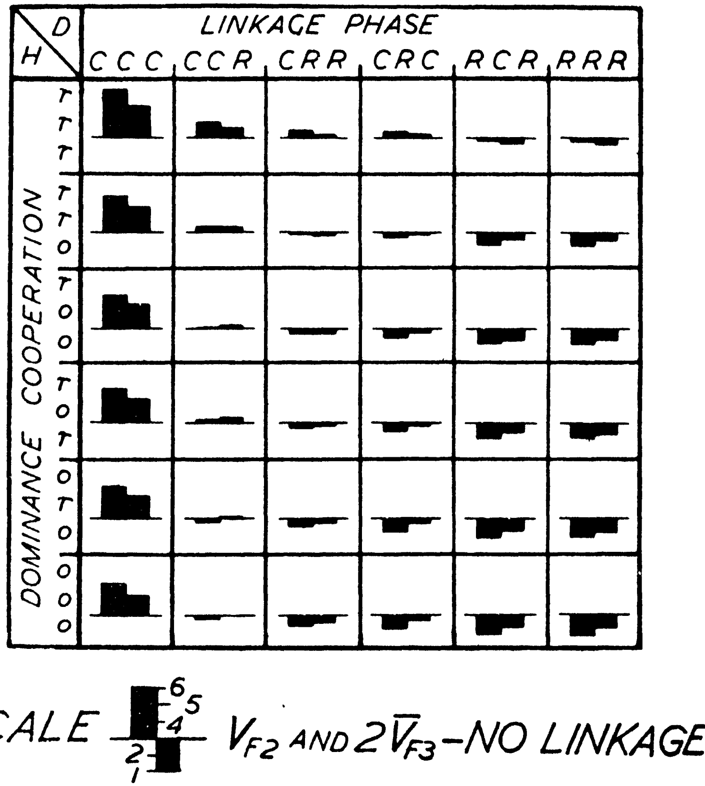


FIG. 13

The effect of linkage phase and dominance cooperation on the contribution made by four segregating genes (all d's and h's assumed to be 1), to V_{F2}, left, and $2\bar{V}_{F3}$, right. The zero line is the value 3, contributed by four genes to both V_{F2} and $2\bar{V}_{F3}$ when they are unlinked Values above 3 are represented by solids above, and those below three by solids below the line, as indicated on the scale The value for $2\bar{V}_{F3}$ is generally, but not always, between that for V_{F2} and the unlinked value of 3. Adjacent genes are assumed to show $p=0{\cdot}125$ with no interference. C=coupling, R=repulsion, r=reinforcement, o=opposition These relations are shown for adjacent genes so that, for example, CRC indicates $\frac{\mathbf{ABcd}}{\mathbf{adCD}}$. Similarly *oro* indicates that h_a and h_b have signs similar to one another but opposite to those of h_b and h_c, where **a**, **b**, **c**, **d** is the order in the chromosome

Curiously enough with this tight linkage, the maximum change relative to the value of V_{F2} in the repulsion series, and hence the

easiest detection of the linkage, does not come with adjacent genes repulsed and opposed. It is in fact obtained with the arrangement *RCR* and *oro* where *R* indicates repulsion, *C* coupling, *o* opposition and *r* reinforcement of adjacent genes, the four genes being treated as three successive pairs. But at $p=0{\cdot}250$ the maximum change is found with *RCR* and *ooo*, and at $p=0{\cdot}375$ with the extreme arrangement *RRR* and *ooo*. On the coupling and reinforcement side, of course, the extreme situation *CCC* and *rrr*, always gives the greatest variance drop.

It is clear, therefore, that although a significant difference between V_{F2} and $2\bar{V}_{F3}$ will give evidence of linkage, the phasic balance cannot be inferred with full certainty from the directions of the change between F_2 and F_3 when dominance is present and the change is small. If D and H were assessed separately in F_3, as they are in F_2, the situation would become much clearer, for any counteracting effects that their changes might have relative to one another, would then become apparent. Such a separation could be made if F_4 data were available to do for F_3 what F_3 data do for F_2.

17. THE TEST OF LINKAGE

Basically the test of linkage is a test of homogeneity of D and H over F_2 and F_3 or over 1B and 2B, or over both sets of data if available simultaneously. No data are, however, available to illustrate the test as applied to backcrosses. F_2 and F_3 data will be used to show how it can be carried out.

Quisenberry's oat data (Table 11) will serve for the purpose. V_{F2}, $V_{\overline{F3}}$ and $W_{F2/F3}$ are available for the estimation of the D and H of the F_2 generation, while $\bar{V}_{F3}$ involves D and H of the F_3 generation. Now if we assume that D_{F3} and H_{F3} may differ from D_{F2} and H_{F2} respectively, a perfect fit must be obtained for $\bar{V}_{F3}$. In fact, adjustment in either D or H alone will secure this perfect fit. Thus D_{F3} and H_{F3} cannot be assessed separately. Nor is there any necessity to assess them separately for the purpose of a linkage test: the important thing is that as a result of such adjustment the sum of squares of deviations of the observed statistics from their expectations, based on the best fitting D's and H's, will be reduced by this perfect fit in $\bar{V}_{F3}$. And since only one adjustment is necessary to achieve this fit, only one degree of freedom is used up in separating D_{F3} and H_{F3} from D_{F2} and H_{F2}. So our test here devolves itself into estimating D and H from V_{F2}, $V_{\overline{F3}}$ and $W_{F2/F3}$ alone, assuming a perfect fit in $\bar{V}_{F3}$. The residual sum of squares is then calculated and compared with the sum of squares of

deviations from expectation when a single D and a single H are estimated from all the data together. The comparison shows us how far the assumption of linkage reduces the sum of squares of deviation, and so enables us to judge whether the effect is a significant one.

Part of the work for the linkage test, the estimation of D and H from the whole data, has already been done in Section 13. We must now undertake a similar calculation with $\bar{V}_{F3}$ excluded. We then have

$$V_{F2}=\tfrac{1}{2}D+\tfrac{1}{4}H+E_1 =1\cdot3158$$
$$V_{\bar{F3}}=\tfrac{1}{2}D+\tfrac{1}{16}H+E_2=0\cdot8809$$
$$W_{F2/F3}=\tfrac{1}{2}D+\tfrac{1}{8}H =0\cdot7161$$
$$E_1=0\cdot3427$$
$$E_2=0\cdot0495$$

E_3 vanishes with the exclusion of $\bar{V}_{F3}$, as a perfect fit must also be secured for E_3 by the adjustment of D_{F3} and/or H_{F3}.

Four least squares expressions may then be found for the estimation of D, H, E_1 and E_2, by the same method as before. These expressions are

$$0\cdot750000D+0\cdot218750H+0\cdot500000E_1+0\cdot500000E_2$$
$$0\cdot218750D+0\cdot082031H+0\cdot250000E_1+0\cdot062500E_2$$
$$0\cdot500000D+0\cdot250000H+2\cdot000000E_1$$
$$0\cdot500000D+0\cdot062500H \qquad +2\cdot000000E_2$$

Inversion of this least squares matrix by equating these expressions to 1, 0, 0, 0, &c., and solving, gives the c matrix of Table 23.

TABLE 23

D	H	E_1	E_2
c_{DD} 10·526316	c_{HD} −30·315789	c_{1D} 1·157895	c_{2D} −1·684210
c_{DH} −30·315789	c_{HH} 107·789473	c_{1H} −5·894737	c_{2H} 4·210526
c_{D1} 1·157895	c_{H1} −5·894737	c_{11} 0·947368	c_{21} −0·105263
c_{D2} −1·684210	c_{H2} 4·210526	c_{12} −0·105263	c_{22} 0·789474

And since $S(Dy)=1\cdot4564$ the c matrix gives $D=1\cdot3294$

$S(Hy)=0\cdot4735$ $H=1\cdot0275$

$S(E_1y)=1\cdot6585$ $E_1=0\cdot3685$

$S(E_2y)=0\cdot9304$ $E_2=0\cdot1007$

From these the expectations for V_{F2}, &c., may be found as in Table 24.

TABLE 24

Grain length in oats

	Expectations	Deviations	
V_{F2}	1·2901	−0·0933	0·1447
V_{F3}	0·8296	0·0227	0·0799
$W_{F2/F3}$	0·7931	−0·1317	−0·0224
E_1	0·3685	−0·0550	0·0034
E_2	0·1007	−0·0564	−0·0461
$\bar{V}_{F3}$	0·7878	0·0191	−0·0191
E_3	0·3100	−0·0256	0·0256

The differences between these expectations and the values observed in the two halves of the experiment (see Table 11) are shown against the corresponding expectations. It will be noticed that $\bar{V}_{F3}$ and E_3 have deviations entered against them. The estimation of the D's, H's, &c., gave perfect fits with the mean values of these two statistics, but the deviations are introduced by the departures of these quantities in the two halves of the experiment from their means. The sum of squares of all the deviations is 0·064772 as compared with a sum of squares of 0·065005 obtained previously. Thus making allowance for linkage has reduced the sum of squares by only 0·000233. As we have seen, 1 degree of freedom is used up in this allowance for linkage, leaving 8 of the original 9 for the residual sum of squares. The linkage mean square, 0·000233, is therefore less than the remainder, or error, mean square of 0·064772/8, i.e. 0·008097. There is no evidence of linkage.

The remainder sum of squares can be analysed further. The differences between the duplicate observations of the seven quantities, V_{F2}, &c., from the two halves of the experiment, account for a sum of squares of 0·039730. This will correspond to 7 degrees of freedom, and the full analysis of variance then becomes:

Item	S.S.	N	M.S.	$t_{[7]}$	P
Linkage	0·000233	1	0·000233		
Residual Interaction	0·025042	1	0·025042	2·10	0·10–0·05
Duplicates	0·039730	7	0·005676		
Total	0·065005	9			

The item for Residual Interaction measures the variation in D and H between V_{F2}, $\bar{V}_{F3}$ and $W_{F2/F3}$, such as would be produced by genic interaction. It gives a $t_{[7]}$ of 2·10 when compared with the estimate of error based on differences between duplicate observations. Such a t is just not significant and so there is neither evidence of linkage nor trustworthy evidence of any other disturbance in D and H.

The *Petunia* corolla length data of Table 13 can be tested for linkage in the same way. Here a perfect fit to $\bar{V}_{F3}$ does not also remove a non-heritable item from the analysis, since both $\bar{V}_{F3}$ and V_{F2} contain E_1. The c matrix for the estimation of D and H excluding $\bar{V}_{F3}$ is therefore the same as that used for the oat data, even though the latter originally included an extra environmental component E_3. Using this same c matrix (Table 23), and conducting the analysis in the way used for the oats, linkage is found to account for a sum of squares of 0·001756 out of the total sum of squares of 0·004241 found in Section 13. We have seen that the total corresponds to 2 degrees of freedom, so that the remainder, 0·002485, left over after allowing for linkage, has only 1 degree of freedom. This corresponds to the Residual Interaction item of the oat analysis, the Duplicates item being missing because all observations were unique. The test of linkage is clearly very insensitive in this case, but since the linkage mean square is less than the remainder mean square, there is no valid evidence of disturbance from this cause. We shall, however, see later that linkage was most likely in operation. It may well be that some residual genic interaction was present too. This would not be surprising in a species cross.

The data on abdominal chaetae number in *Drosophila*, also considered in Section 13, require a different c matrix in the linkage test, since the third generation families were biparentals, not F_3's. In this case $\bar{V}_{BIP}$ must be excluded from the estimation of D and H, since linkage would permit a separate adjustment of D and H in $\bar{V}_{BIP}$ corresponding to the separate adjustment in $\bar{V}_{F3}$. The least squares matrix from Table 15, excluding $\bar{V}_{BIP}$, is

$$
\begin{array}{llll}
0{\cdot}375000D+0{\cdot}140625H+0{\cdot}500000E_1+0{\cdot}250000E_2 \\
0{\cdot}140625D+0{\cdot}066406H+0{\cdot}250000E_1+0{\cdot}062500E_2 \\
0{\cdot}500000D+0{\cdot}250000H+2{\cdot}000000E_1 \\
0{\cdot}250000D+0{\cdot}062500H \qquad\qquad\quad +2{\cdot}000000E_2
\end{array}
$$

the inversion of which gives Table 25.

TABLE 25

D		H		E_1		E_2	
c_{DD}	14·315790	c_{HD}	−30·315790	c_{1D}	0·210526	c_{2D}	−0·842105
c_{DH}	−30·315790	c_{HH}	94·315790	c_{1H}	−4·210526	c_{2H}	0·842105
c_{D1}	0·210526	c_{H1}	−4·210526	c_{11}	0·973684	c_{21}	0·105263
c_{D2}	−0·842105	c_{H2}	0·842105	c_{12}	0·105263	c_{22}	0·578948

And since $S(Dy) = 3·7293$ we find $D = 1·5263$

$S(Hy) = 1·7336$ $H = -2·9323$

$S(E_1y) = 13·1719$ $E_1 = 6·5709$

$S(E_2y) = 2·4690$ $E_2 = 1·1353$

compared with $D=0·7823$, $H=-0·6166$, $E_1=6·6642$, $E_2=1·1560$ found when no allowance was made for linkage. The deviations of observation from expectation are now much smaller than before as Table 26 shows.

TABLE 26

Abdominal chaetae in *Drosophila*

Statistic	Observed	Expectations		Deviations	
		Inclusive	Exclusive	Inclusive	Exclusive
V_{F2}	6·6214	6·9012	6·6010	−0·2798	0 0204
$V_{\overline{BIP}}$	1·2518	1·3130	1·3336	−0·0612	−0·0818
$W_{F2/BIP}$	0·4227	0·1956	0 3816	0·2271	0·0411
$\overline{V}_{BIP}$	7·1377	6·7442	7·1377	0·3935	0·0000
E_1	6·5505	6·6642	6·5709	−0·1137	−0·0204
E_2	1·2172	1·1560	1·1353	0·0612	0·0819

'Inclusive' items are based on the inclusion of $\overline{V}_{BIP}$ as in Section 13.
'Exclusive' items are based on the exclusion of $\overline{V}_{BIP}$ as in this section.

The sum of squares of inclusive deviations differs from that of the exclusive deviations by the linkage item, which has 1 degree of freedom. As in the *Petunia* case, the inclusive sum of squares is based on 2 degrees of freedom. The remainder or error sum of squares derived directly from the exclusive deviations will, therefore, have 1 degree of freedom. The analysis of variance thus becomes

Item	S.S.	N	M.S.	$t_{[1]}$	P
Linkage	0·289203	1	0·289203	4·26	0·20–0·10
Remainder	0·015920	1	0·015920		
Total	**0·305123**	**2**			

A t for only 1 degree of freedom requires to be very large before it is significant. Consequently there is no trustworthy evidence of linkage in the present case, even though the linkage mean square is some 18 times as large as the remainder. It is, however, of interest that this suspicion of linkage, which if it exists must be in the repulsion phase since $V_{F2} < \bar{V}_{B1P}$, is borne out by the behaviour of the flies under selection. Mather (1941) found it necessary to assume linkage in the repulsion phase to account for his selection results after the fourth and fifth generations. The present test strongly suggests, even if it does not prove, that such linkage was already displayed in the third generation.

18. COUPLING IN BARLEY (in collaboration with Dr. U. Philip)

In 1941 an experiment was conducted on the inheritance of ear conformation in the cross between Spratt and Goldthorpe barley. The metric used was the discriminant function, compounded of ear width, total ear length, and length of the central six internodes of the ear, whose calculation has already been described in Section 6. In design the experiment consisted of five randomized blocks, each containing 117 plots. Of these, 100 were devoted to 100 different F_3 families from the F_2 plants of 1940 (taken from those used in calculating the discriminant function), 10 were devoted to F_2 plants, 3 to F_1 plants, and 2 to each parental variety. The plots were intended each to contain 10 plants, but owing to adverse circumstances this number was not reached in every case. Indeed, all ten plants or nine out of ten failed in a few plots. These plots were excluded from the analysis. The blocks differed owing to the effects of soil fertility on ear conformation, so that all calculation of variances and covariances were made using deviations from the plot mean, for V_{F2} and $\bar{V}_{F3}$, and the block mean, for $V_{\overline{F3}}$ and $W_{F2/F3}$ (as opposed to the general mean) for each family. Sums of squares and sums of cross-products of deviations from these means were pooled for all blocks to give the total sums of squares and cross-products for the whole experiment.

The inclusion of parents and F_1 several times in each block permits an empirical determination to be made of E_2, the non-heritable variance of plot means round the block mean. This non-heritable variance of plot means is a component of $V_{\overline{F3}}$. The non-heritable variance of plants within a plot, E_1, necessary for the partition of V_{F2} and $\bar{V}_{F3}$, was also estimated from the parent and F_1 plants in the experiment, the sums of squares of parental

and F_1 plants round the plot means being pooled over the block and finally over the experiment as a whole.

The mean of all Parent 1 (P_1) plot means, was 327·9, and that of P_2 was 300·6. The F_1 showed heterosis with a mean of 400·2, and the F_2 mean was 362·3. This recession of F_2 mean towards the mid-parent value of 314·2 was, of course, expected; and on this evidence alone the scale of the discriminant function seems fairly satisfactory, as the recession should be to a point half-way between $\bar{F}_1$ and mid-parent, viz. to 357·2. The mean of all F_3 means (343·7) was, however, also somewhat higher than expected, whether on the basis of a three-quarter's recession from $\bar{F}_1$ to mid-parent (335·7 expected) or on the basis of a half recession from $\bar{F}_2$ to mid-parent (338·3 expected). It would appear, therefore, that the scale is probably somewhat exaggerated at its upper end, and this view is confirmed when the F_3 data are examined more closely. The lowest mean among the F_3 families was 181·2, a departure of 133·0 from the mid-parent. Ten families out of the hundred departed equally or in greater measure from the mid-parent in the opposite direction, and three departed by over 186. There can thus be little doubt of the deficiencies in the scale, but it was not felt that the resulting distortion of the data would be sufficiently serious to warrant the labour of re-calculation after transformation to a more satisfactory metric.

The various second degree statistics are given separately for the five blocks of the experiment in Table 27, together with their values obtained by pooling over all blocks. These joint values approximate to the means of the individual block values, but do not precisely equal them. The small discrepancies arise because the joint values were obtained by pooling the sums of squares and cross products over all the blocks and so arriving at a joint value weighted according to the contributions of the various blocks. The weights of the various blocks were not quite equal because of the failure of certain plots in the experiment, as already noted.

The analysis of the joint estimates of the statistics was conducted with the inclusive (including $\bar{V}_{F3}$) and exclusive (excluding $\bar{V}_{F3}$ so as to make allowance for the effects of linkage) c matrices used for the *Petunia* corolla length experiment (Tables 14 and 23), which the present experiment clearly resembles in its structure. The values of $S(Dy)$, &c., and the estimates of D, H, E_1 and E_2 derived from them, are given in Table 28, for both the inclusive and exclusive cases. From these the inclusive and exclusive expectations were found for V_{F2}, &c., as shown in Table 27 and

Fig. 14. The analysis of variance, derived from the sums of squares of deviations of the separate block estimates of these statistics from the expectations, is set out in Table 29.

TABLE 27

Barley Data—Ear Conformation

Statistic	Block A	Block B	Block C	Block D	Block E	Joint	Expectations Inclusive	Expectations Exclusive
V_{F2}	9492·0	11540·9	9179·5	8673·7	9926·4	9713·1	9326·2	9710·6
$V_{\overline{F3}}$	6289·7	5838·6	5935·1	7148·8	6013·4	6246·7	6241·6	6241·6
$W_{\overline{F2}\cdot F3}$	6934·6	6342·5	6742·5	7439·6	6696·8	6833·0	6840·5	6840·5
$\bar{V}_{F3}$	4513·1	4532·5	4342·4	4323·1	3867·9	4314·1	5082·9	4314·1
E_1	1443·8	736·9	1433·1	1720·9	747·6	1221·4	839·6	1224·0
E_2	278·6	57·9	306·8	403·2	54·9	219·1	224·2	224·2

TABLE 28

Results of Analysis of Barley Data

	Inclusive	Exclusive		Inclusive	Exclusive
$S(Dy)$	12474·9	11396·4	D	10388·8	10388·8±808·9
$S(Hy)$	4212·1	3672·8	H	13169·0	13169·0±2588·4
$S(E_1y)$	15248 7	10934·6	E_1	839·6	1224·0±242·7
$S(E_2y)$	6465·8	6465·8	E_2	224·2	224·2±221·5

TABLE 29

Analysis of Variance of Barley Data

Item	S S.	N	M.S.	$t_{[25]}$	P
Linkage	4590309	1	4590309	3·76	0·001
Residual Interaction	14329	1	14329	0·21	0·90–0·80
Replication	7755458	24	323144		
Total	12360096	26			
Pooled Error	7769787	25	310791		

Six statistics were found from each of five blocks, so that there are 30 deviations in all, giving 30 degrees of freedom. Of these 4 are taken up in fitting D, H, E_1 and E_2, leaving 26. Since there are 5 estimates of each statistic, each row of Table 27 contributes 4 degrees of freedom, to a pool of 24, for differences between replicate estimates. The remaining 2 of the 26 degrees of freedom appearing in the analysis are assignable, 1 to the effect of Linkage and 1 to the variation in D and H of the kind ascribable to residual genic interaction. This latter item is therefore labelled Residual Interaction in the analysis. It is clear that there is good evidence

of linkage, but no evidence of any other variation in the values of D and H. The shortcomings of the scale have failed to inflate seriously the item for Residual Interaction. These shortcomings cannot therefore be having any material effect on the value of D and H, though as we shall see, they affect the use of these quantities in predicting advances under selection.

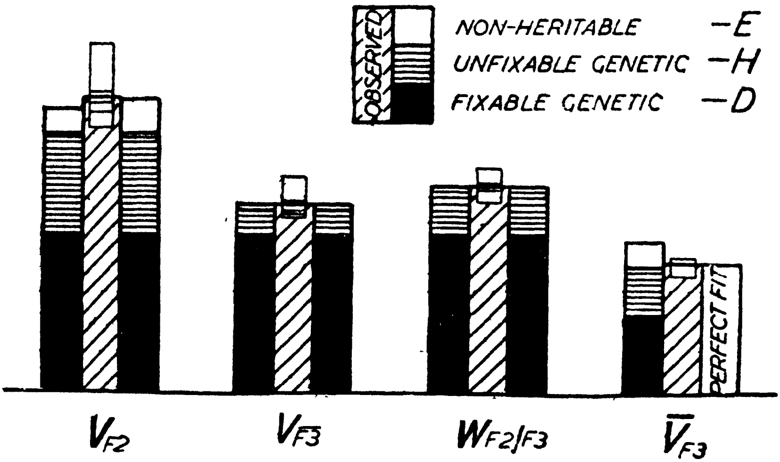


FIG. 14

BARLEY : EAR CONFORMATION VARIATION

The partition of variability for ear conformation in barley. The centre column shows the pooled value observed for each statistic, the differences between the five replicates being indicated by the box, and the lines across it, at the top Less than five values may be indicated in the box where two or more replicate gave the same value, or where one or more values coincide with the pooled value The left columns show the values expected, and the composition of these values when D and H are estimated assuming no linkage. The right columns show the same when D and H are estimated making allowance for linkage by assuming a perfect fit for $\bar{V}_{F3}$. Linkage is present

The value of $\bar{V}_{F3}$ is less than $\frac{1}{2}V_{F2}$ even before E_1 has been taken from each, and so there has been a fall in D and H from F_2 to F_3. This may well be due, in part, to the linked genes having reinforcing actions in the heterozygotes, but since H contributes less to V_{F2} and $\bar{V}_{F3}$ than does D, it seems inescapable that the change must be largely due to the linkage being preponderantly in the coupling phase, that is to say, within the linked group or groups the + allelomorphs must be preponderantly associated with one another in the chromosome from one parent and the − allelomorphs in the other. The means of the two parents do not however differ widely. It must therefore be supposed further that at least

two linked groups of genes are involved, coupling existing within each group, but the two groups balancing one another in the parents. Each parent has the + group of allelomorphs in one chromosome together with the - group of allelomorphs of the other.

When the effect of linkage has been removed the remaining 25 degrees of freedom (which may be pooled once the interaction item has shown itself not to be significant) give an error mean square of 310791·5. From this the standard errors of D and H, as they appear in of course the F_2 generation, may be found in the way already described (Section 13), but using c_{DD}, &c., from the exclusive matrix (Table 23). There will be a factor of $\frac{1}{5}$ in the calculation because the experiment contained 5 replicates. These standard errors are given in Table 28. It will be seen that though s_H is 2588·4, the value of H itself is 5·09 times as high. A $t_{[25]}$ of 5·09 has a probability of less than 0·001, and so it can hardly be doubted that H is significantly greater than 0. Thus the genes determining ear conformation show dominance, and since the F_1 shows heterosis this dominance must be preponderantly in the direction of high values. H is actually larger than D, but the difference is obviously not significant, and so there is no ground for postulating superdominance, i.e. for postulating that $h > d$ for any gene.

Before leaving this example it should be noted that the estimate of E_2 has a standard error nearly as large as itself. This somewhat suspicious result is a consequence of the method of analysis which gives equal weight to all the statistics, V_{F2}, &c., used in the estimation of D, H, E_1 and E_2. The variation in the direct estimate of E_2 is, of course, actually less than that in V_{F2} and the other statistics of larger values. The standard error of E_2 arrived at from the unweighted analysis may well therefore be somewhat too high. Weighting would give a more reliable value, but the extra work entailed would hardly be justified by the extra precision achieved in assessing the reliability of the estimate of E_2. The values of D and H are of much more importance and since the estimates of these are affected by the values of all the statistics used, the standard errors found for them should be more trustworthy.

CHAPTER 6

THE NUMBER OF EFFECTIVE FACTORS

19. THE SOURCES OF ESTIMATES

THE PARTITION of variation into the D, H and E components show us how much of it is (*a*) heritable and fixable in the form of differences between homozygotes, (*b*) heritable but unfixable in that it depends on differences between heterozygotes and the means of the corresponding pairs of homozygotes, and (*c*) non-heritable and hence merely serving to obscure the genetical situation. The test of linkage enables us to go further and foresee the likelihood, the extent, and the direction of change in the heritable components of variation in the next succeeding generations. One further piece of information is, however, required before the full import of the heritable components of variation can be assessed, viz. the number of genes or units of inheritance contributing to the D and H components.

If the D component depends on the difference produced by one gene only, its distribution among F_3 families, for example, will be simple. Half the families will be homozygous and hence will segregate no further. The other half will be heterozygous and will repeat the behaviour of F_2. Even in limited groups of F_3's the full potentialities of segregation will be realized. If, on the other hand, D is composed of items contributed by 10 unlinked genes, only rarely will a true breeding family be obtained in F_3, and many grades of segregation will be encountered among the various families. To this question of determining the number of genes involved we must now turn, and in answering the question we shall see that the gene is in fact no longer the unit of inheritance that we must use.

Where all the + allelomorphs of the k genes, whose differences are involved in the cross, are concentrated in one of the true breeding parent lines, and all the − allelomorphs in the other, half the difference between the two parental means, i.e. the deviation of either parent from the mid-parent value, will supply an estimate of $S(d_a)$. If all these genes give equal increments, i.e. $d_a = d_b = \ldots = d$, $S(d_a) = kd$. Furthermore, when this is true, $d_a^2 = d_b^2 = \ldots = d^2$ and $D = S(d_a^2) = kd^2$ in the absence of linkage. Thus the ratio which the square of half the parental difference bears to D is $\frac{S^2(d_a)}{S(d_a^2)} = \frac{(kd)^2}{kd^2} = k$.

This method of estimating k is the one used by Sewall Wright (1934), except that in the absence of F_3 data Wright could not separate H from D, and so was forced to use an inflated value of the denominator. Charles and Goodwin (1943) and Goodwin (1944) have also used this method of estimation for a number of cases, and have indeed given a formula (though without showing the derivation), based on the same assumptions, for estimating the minimal number of genes common to two polygenic segregations. The effect of dominance in lowering the estimate of k has been discussed by Serebrovsky (1928).

Leaving the question of linkage aside for the moment, this estimate of k may be distorted in either or both of two ways even when D has been freed from H. The genes may not all give equal d increments, and the + and − allelomorphs may not be distributed isodirectionally between the parents. Both inequality of the d increments and incomplete concentration of like allelomorphs in the parents must lead to a spuriously low estimate of k. Let us consider first the effect of inequality. We may set

$$d_a=d(1+\alpha_a),\ d_b=d(1+\alpha_b),\ .\ .\ .,\ d_k=d(1+\alpha_k);$$

where d is the mean increment. Then

$$S(\alpha_a)=0, \quad S(d_a)=kd \quad \text{and} \quad S^2(d_a)=k^2d^2$$

But

$$D=S(d_a^2)=S[d^2(1+\alpha_a)^2]=d^2S(1+\alpha_a)^2$$
$$=d^2[k+2S(\alpha_a)+S(\alpha_a^2)]$$

Now since $S(\alpha_a)=0$, $S(\alpha_a^2)=kV_\alpha$, where V_α stands for the variance of α. Thus $D=kd^2(1+V_\alpha)$, and K_1, the estimate of k found as the ratio borne by the square of half the parental difference to D, is therefore

$$K_1=\frac{k^2d^2}{kd^2(1+V_\alpha)}=\frac{k}{1+V_\alpha}$$

Since V_α cannot be negative, $K_1<k$ except in the special case of equality of all the d increments. With such equality

$$V_\alpha=0 \quad \text{and} \quad K_1=k$$

The effect of incomplete concentration will be to reduce the difference between the parental means below its maximum of $2S(d_a)$. The value of D is, however, unaltered, and in consequence the estimate, K_1, must in such a case be less than k. With a perfect balance of + and − genes, the two parents will be alike, and K_1 must take the value of O.

An estimate of k may be reached in a similar way, but making

use of the h increments in place of the d's. If all the genes have reinforcing h increments, the departure of the F_1 mean from the mid-parent value will be $S(h_a)$, which when $h_a=h_b= \ldots =h$, is kh. Then the square of this departure divided by H, which in the absence of linkage is defined as $S(h_a^2)$, will give an estimate of k. Just as with the estimate based on the d increments, this new estimate will be reduced by any inequality of the h's and also by any opposition of the h's.

These two methods of estimating k are similar in principle, and in consequence they have the same disadvantages. A further method of estimation has, however, been proposed by Panse (1940a and b), which overcomes the difficulties of incomplete concentration and incomplete reinforcement. Half the individuals in F_2 are expected to be homozygous for one or other allelomorph of any gene which is segregating. This gene will, therefore, contribute nothing to the heritable variance of the F_3's obtained from such individuals. The remaining F_2 individuals will be heterozygous for the gene, and it must therefore contribute $\frac{1}{2}d^2+\frac{1}{4}h^2$ to the heritable variance of their F_3's. Let us denote $\frac{1}{2}d_a^2+\frac{1}{4}h_a^2$ by x_a. Then the contributions of **A-a** to the mean variance of F_3 is, as we have already seen, $\frac{1}{2}(\frac{1}{2}d_a^2+\frac{1}{4}h_a^2)$ or $\frac{1}{2}x_a$. But the variance of the F_3 variances (V_{VF3}) will, so far as this gene is concerned, be $\frac{1}{2}x_a^2-(\frac{1}{2}x_a)^2$ or $\frac{1}{4}x_a^2$. Now with independent inheritance and independent action, the contributions of the various genes will be additive and hence ${}_H\bar{V}_{F3}$ will be $\frac{1}{2}S(x_a)$ and ${}_HV_{VF3}$ will be $\frac{1}{4}S(x_a^2)$. Then where $x_a=x_b= \ldots =x$, ${}_H\bar{V}_{F3}=\frac{1}{2}kx$ and ${}_HV_{VF3}=\frac{1}{4}kx^2$, so that $k=\frac{{}_H\bar{V}_{F3}^2}{{}_HV_{VF3}}$, the sub-$H$ in front of V indicating that it is the heritable portion of the variance which is under consideration. Where $x_a\neq x_b \ldots \neq x_k$ we can set $kx=S(x_a)$ and $x_a=x(1+\beta_a)$, $x_b=x(1+\beta_b)$, &c. Then our estimate of k is

$$K_2=\frac{k}{1+V_\beta}$$

As an estimate of k, K_2 is superior to K_1 in not being subject to reduction by incomplete concentration. Since, however, $x_a=\frac{1}{2}d_a^2+\frac{1}{4}h_a^2$, the variation in β measured by V_β will be greater than the variation in α measured by V_α. This is partly because V_β measures the variation of d_a^2 as opposed to d_a, and partly because V_β must be inflated by any variation in the ratio $\frac{h}{2d}$.

It may be noted in this latter connection that an estimate of k, similar to K_2, can be obtained from the variances of the second

backcrosses. Where ${}_H\bar{V}_{2B}$ stands for the sum of the heritable variances of the two second backcrosses (to the two parental lines) of a single first backcross plant, we find that

$${}_H\bar{V}_{2B}={}_H\bar{V}_{B11}+{}_H\bar{V}_{B12}={}_H\bar{V}_{B21}+{}_H\bar{V}_{B22}=\tfrac{1}{4}S(d_a^2+h_a^2),$$

the mean being taken over the variances of the progenies of all the plants in the first backcross. Similarly ${}_HV_{V2B}=\frac{1}{16}S(d_a^2+h_a^2)^2$. Then we can estimate k as $\frac{{}_H\bar{V}^2_{2B}}{{}_HV_{V2B}}$ and the reduction in value due to inequality of $(d_a^2+h_a^2)$, $(d_b^2+h_b^2)$, &c., will depend on the variation in value of d^2, in the same way as with K_2, but also on the variation in $\frac{h}{d}$, not $\frac{h}{2d}$ as with K_2. If this new estimate is lower than K_2 the difference must then be ascribed to the greater effect of variation in $\frac{h}{d}$, which should exceed in general the variation in $\frac{h}{2d}$ through which K_2 is reduced. This comparison, therefore, provides a test, though probably not a sensitive test, of the variation in the proportionate dominance, i.e. the ratio borne by the h increment to the d increment, from gene to gene. The means of estimating k from backcross data is of course especially valuable with animals and dioecious plants where F_3 families cannot be raised.

If F_4 data are available, the lowering effect of variation in the $\frac{h}{2d}$ ratio can be avoided. Each F_2 individual can be regarded as an F_1 and the D and H increments separated within its descendance by the means already described. In this way we should have the value of D available separately for each F_3 family, and the process of estimation could proceed using D and V_D in place of ${}_H\bar{V}_{F3}$ and ${}_HV_{VF3}$, so eliminating the distracting influence of H. The process could also, of course, be carried out with $\bar{H}$ and V_H, and the discrepancy between the two estimates would reflect the difference between the magnitudes of variations of d and h increments.

Even, however, when the consequences of variation in h/d have been avoided, K_2 will be less than k by an amount depending on V_β. Now if V_α is small, $V_\beta \simeq 4V_\alpha$; but if V_α is not small (or, of course, if variation in h/d is having its effect), $V_\beta > 4V_\alpha$. In either case $K_2 < K_1$, unless $V_\beta = V_\alpha = 0$ when $K_2 = K_1 = k$, or unless the K_1 is reduced by incomplete concentration of allelomorphs in the parents.

Where the distribution of allelomorphs is isodirectional in the

parental lines so that K_1 is not reduced in value from lack of full concentration, we have

$$k = K_1(1+V_\alpha) = K_2(1+V_\beta)$$

Then putting
$$r = \frac{V_\beta}{V_\alpha}$$

$$V_\alpha = \frac{K_1 - K_2}{rK_2 - K_1}$$

and
$$k = \frac{K_1 K_2 (r-1)}{rK_2 - K_1}$$

When V_α is small and $r=4$

$$V_\alpha = \frac{K_1 - K_2}{4K_2 - K_1} \quad \text{and} \quad k = \frac{3K_1 K_2}{4K_2 - K_1}$$

To assume that $r=4$ when V_α is not small would lead to an under-estimate of k, a negative value being obtained where $K_1 > 4K_2$.

Thus when both K_1 and K_2 are available, something may be learned of the variation in magnitude of d from gene to gene; or if the estimate based on the departure of F_1 from mid-parent is used in place of K_1, we learn something of the variation of h.

When, as judged by comparison with K_2, a useful value of K_1 or of its counterpart is not available, K_2 may still be put to good use. It is then possible to calculate from K_2 and D the difference between two true breeding strains which would give a K_1 equal to the observed K_2, i.e. to calculate the difference between two strains which would respectively contain all the + and all the − allelomorphs. Since the mid-parent value is independent of the concentrations of allelomorphs, the mean measurements of these two hypothetical strains could be estimated by adding and subtracting half their difference from the mid-parent, and they would represent the limits to which selection could push the measurement in either direction. Since in general $K_2 < K_1$, these estimates must be minimal. They would be exceeded by even more if some linkage in repulsion, so tight as to have escaped detection, were broken by a fortunate recombination in one of the later generations. Nevertheless, such minimal selective limits would have their use in showing the immediate selective progress that could be expected; for if these limits lay outside the actual parental range, selection would clearly be capable of leading to real progress. If they fell inside the parental range, then equally clearly the concentration of allelomorphs in the two parents would be such that selection would

be unlikely easily to produce anything markedly transgressing what is already available. If selection were judged to be worth while, and the experiment was continued in a form permitting in each generation the calculation of D and K_2 (both of which must be diminishing), the prospects of further advance could be estimated at each stage. The selection could then be discontinued either when D and K_2 fell to zero, or when the prospective progress ceased to be worth while.

Though K_1 and K_2 are estimates of k, they cannot be consistent estimates unless d and h are the same for all genes. Any variation in these increments will lead to underestimation of k. A second limitation of the methods of estimating k is imposed by the assumption that the genes contribute to the heritable variance independently of one another. Now their contributions will not be independent unless the genes recombine freely, and the validity of the estimates must consequently be conditional on the linkage test revealing no evidence of dependent segregation.

If two genes are in fact linked they will appear as less than two in the contribution they make to the variation. With recombination in the region of 0·10 to 0·30 we have a good chance of detecting the linkage and adjusting our estimates of k accordingly. With a higher recombination frequency, the linkage may escape detection. In that case, however, the departure from independence of the contributions made by the genes to the variance will be smaller, and the disturbance in estimating k will not be serious. But tight linkage may also escape detection, and the two genes will then appear more nearly as one in the segregation. Under such circumstances the true number of genes may be virtually impossible to find. Nor need we concern ourselves unduly about the actual number of genes. Statistically, less error is involved in treating a closely linked pair as one unit than as two.

The same essential difficulty is of course encountered in Mendelian genetics when no recombination occurs between two genes distinguishable by their different effects on the phenotype. They must then be treated as one gene of pleiotropic action. The present case differs only in that rare cases of recombination cannot be recognized individually. To be detected, recombination must be sufficiently frequent for its statistical consequences to be clear, and it must be quite free if the genes are to be recognized as fully distinct in their contributions to the variation. This difference in the level of the recombination frequencies, necessary for the recognition of the genes as distinct units in Mendelian and biometrical

genetics respectively, must however mean that genes which would be separated in Mendelian genetics may fall within the same effective unit of inheritance in biometrical genetics. The biometrical units need not therefore be ultimate genes. In consequence they will be referred to by the more empirical term of effective factors. The consequences of linkage and the nature of these factors will become clearer as we consider some examples.

20 THE PROCESS OF ESTIMATION

Of the four examples considered earlier, the *Drosophila* data afford no means of estimating K_2, because neither F_3's nor double backcrosses were included in the experiment. The barley figures must be reserved for special consideration as they show marked linkage. This leaves the oat and *Petunia* results as illustrative material.

The mean grain lengths of the two oat varieties, Victor and Sparrowbill, were 16·361 and 11·484 mms. respectively. The deviation of each from the mid-parent value was therefore $\frac{4 \cdot 877}{2}$ or 2·4385. D has already been found to be 1·3211 (Section 13). Hence $K_1 = \frac{2 \cdot 4385^2}{1 \cdot 3211} = 4 \cdot 501$. Since no comparable mean measurement is available for the F_1, no corresponding estimate can be found from the h increments.

The mean variance of F_3 was observed to be 0·7878, but this includes a non-heritable component, which must obviously be eliminated before K_2 can be estimated. The heritable portion of $\bar{V}_{F3}$ is $\frac{1}{4}D + \frac{1}{8}H$, which from Section 13 is $\frac{1}{4}(1 \cdot 3211) + \frac{1}{8}(1 \cdot 0694)$ or 0·4640. The variance of the F_3 variances is, by direct calculation, 0·1022 in one half and 0·0609 in the other half of the experiment, or 0·08154 over all the data. The variance of the F_3 variances will not be affected by the non-heritable components of individual F_3 variances, unless the magnitudes of these components are themselves correlated with genotype in a way not yet detected, or unless the non-heritable agencies are themselves varying widely in effect. No correction for non-heritable effects will therefore be made in the calculations which follow, though the possibility of the variation in non-heritable agencies lowering the estimate of K_2 must be remembered, and should form the subject of experimental investigation. Professor R. A. Fisher has pointed out to me, however, that V_{VF3} must in any case be inflated by a sampling item, since

each F_3 variance is but an estimate whose accuracy depends on the size of the family from which it was calculated. This sampling component will be $\frac{2}{n-1}\bar{V}^2_{F3}$ (Fisher, 1946), and here $\bar{V}_{F3}$ must be taken as including its non-heritable portion, E_3, since this will serve as well on the heritable portion to inflate the sampling error. In this formula n represents the number of plants per F_3 family. The number of plants was not, in fact, constant in Quisenberry's F_3's, and so the harmonic mean of the family sizes, viz. 32·387, must be used. The pooled value observed for $\bar{V}_{F3}$ is 0·7878, so giving a correction of $\frac{2\times0{\cdot}7878^2}{32{\cdot}387-1}$ or 0·03955, which on subtracting from 0·08154, the value observed for V_{VF3}, leaves 0·04199. The correction for sampling variation is much greater than the variance of the variances of the 36 samples of each parental variety grown in the trial (0·005 for Sparrowbill and 0·017 for Victor). Thus the non-heritable portion of the variance seems unimportant as an agent determining the variation of F_3 variance in this case, except in so far as it inflates the sampling correction. It is not, however, clear that this will always be the situation.

With $\frac{1}{4}D+\frac{1}{8}H=0{\cdot}4640$, and $V_{VF3}=0{\cdot}04199$, $K_2=\frac{0{\cdot}4640^2}{0{\cdot}04199}=5{\cdot}127$.

It should be observed, however, that the two halves of the experiment yielded 0·1022 and 0·0609 as estimates of V_{VF3} respectively. After correction these become 0·0607 and 0·0232, which give the somewhat discrepant values of 3·547 and 9·280 for K_2. The joint estimate of 5·127 must therefore be used with some caution. A further study of the causes of such discrepancies would be worth while in adequately designed experiment. It is clear that the magnitude of the correction for sampling variation is here responsible for part of the trouble. In one half of the experiment it is even greater than the corrected residual. The large size of the correction arises partly from the fact that the non-heritable variation is fairly high (constituting nearly half of $\bar{V}_{F3}$), and so has a large inflating effect on the sampling variance of $\bar{V}_{F3}$. Any method of reducing the non-heritable variation would therefore be valuable in improving the precision of the estimate K_2.

K_2 from the pooled data is 5·127 as compared with a K_1 of 4·501. There are apparently some + allelomorphs in the preponderantly − Sparrowbill and some + allelomorphs in Victor. On the face of it, however, the concentration of like allelomorphs cannot be

far from complete and so selection is not likely to produce lines exceeding Victor or falling short of Sparrowbill by much in grain length. In fact $K_2D=5{\cdot}127\times1{\cdot}3211=6{\cdot}7733$, and hence the immediate selective limit would be a departure in either direction of $\sqrt{K_2D}$ or 2·603 mms. from the mid-parent. Since the parents already depart by 2·439 from their mid-value, selection seems likely to be virtually ineffective unless some undetected close linkage in repulsion be broken.

The lowest F_3 mean observed was 11·70 and the highest 16·18, i.e. departures from the mid-parent of 2·22 mms. and 2·26 mms. respectively. These bear out the expectations quite well, because a family homozygous for all five + allelomorphs, or all five − allelomorphs is not likely to occur in a group of 150 F_3's. These two departures of 2·22 and 2·26 are therefore likely to represent families homozygous for four like allelomorphs out of the five factors.

The reasonable close agreement of the extremes F_3 means with the expectations based on K_2 and D suggests that the value of K_2 is not over low, or in other words that V_β is not very large. One is, therefore, lead to conclude that the five effective factors do not differ much amongst themselves in magnitude of action.

A very different situation is, however, met with in the *Petunia* species cross. The two species of *Petunia* had mean corolla lengths of 7·871 and 2·669 log units respectively, giving a mid-parent value of 5·270. Therefore $S(d)=2{\cdot}601$. D has already been found as 0·7258 in Section 13. Thus

$$K_1=\frac{2{\cdot}601^2}{0{\cdot}7258}=9{\cdot}321$$

Since $D=0{\cdot}7258$ and $H=0{\cdot}0361$, the heritable portion of $\bar{V}_{F3}$ is 0·1860. The observed value of V_{VF3} was 0·04009 and of $\bar{V}_{F3}$ was 0·2762. The harmonic mean size of F_3 families was 9·259 so that the correction for sampling variances becomes $\frac{2\times0{\cdot}2762^2}{9{\cdot}259-1}$ or 0·01847, leaving 0·02162 as the corrected V_{VF3}. Then

$$K_2=\frac{0{\cdot}1860^2}{0{\cdot}02162}=1{\cdot}600.$$

This is in striking contrast to $K_1=9{\cdot}321$. The discrepancy may, of course, be due in part to the estimate of V_{VF3} being over high or to the correction for sampling variance being estimated at too low a figure. But even if the correction is arbitrarily increased by 50% K_2 still has a value of less than 3, and the difference between K_1

and K_2 remains large. It seems clear, therefore, that the value of V_β must be high, or in other words that the individual effects of the factors concerned are highly variable. Consequently neither K_2 nor even K_1 can be regarded as a reliable estimate of k. Certainly K_2 affords no suggestion that genotypes can be found to give corolla lengths greater than that of *P. axillaris* or less than that of *P. violacea*. K_1 and K_2 may, however, be used for another purpose.

We have already seen that given the value of $r\left(=\frac{V_\beta}{V_\alpha}\right)$ and knowing K_1 and K_2, we can estimate both V_α and k. With $K_2=1\cdot600$ and $K_1=9\cdot321$, r must be at least $\frac{9\cdot321}{1\cdot600}$, i.e. $5\cdot826$, or k will be estimated as a negative quantity. With $K_2=4$, an arbitrary value which one might choose to consider in case the correction made to V_{1F3} was much too low, r might take any value down to 4, the lower limit set by other considerations (Section 19). Fig. 15 shows the values of k and V_α respectively, given when K_2 is either $1\cdot600$ or $4\cdot0$, when r lies between the fairly extreme value of 10 and its possible minimum. From these figures it appears that with $K_2=4$, k seems likely to lie between 17 and 11, and V_α between $0\cdot8$ and $0\cdot2$. If, however, $K_2=1\cdot600$, k seems unlikely to be much below 20 or V_α below $1\cdot2$. In this latter case both k and V_α could be very large; but even when r exceeds its minimum of $5\cdot826$ by only 1, k is little over 50 and V_α only about $4\cdot5$. Thus k seems unlikely to be less than 11 or more than 50. Furthermore, so low a value as 11 is possible only if K_2 is grossly underestimated. Similarly with $K_2=1\cdot600$, k is rapidly falling away as r increases. If all values of r up to about 10 can be regarded as equally likely *a priori*, k is as likely to be below 30 as above it, and it may well be as low as 20.

In the absence of any further information no more precise conclusion may be drawn, but even these somewhat indefinite findings have their value. If we are to regard the effective factors, of whose number k is an estimate, as the ultimate genes on which the inheritance of corolla length depends, the number 50 would not seem to be any too large. The species are nearly isolated from one another genetically, and they differ markedly in this character. Certainly under these circumstances 11 would seem an unexpectedly small number, and even a number so low as 20 would perhaps be somewhat surprising. The indications are therefore

that k is not a measure of the number of ultimate genes, and this conclusion is also suggested in another way.

The haploid number of chromosomes in both *Petunia* species is 7

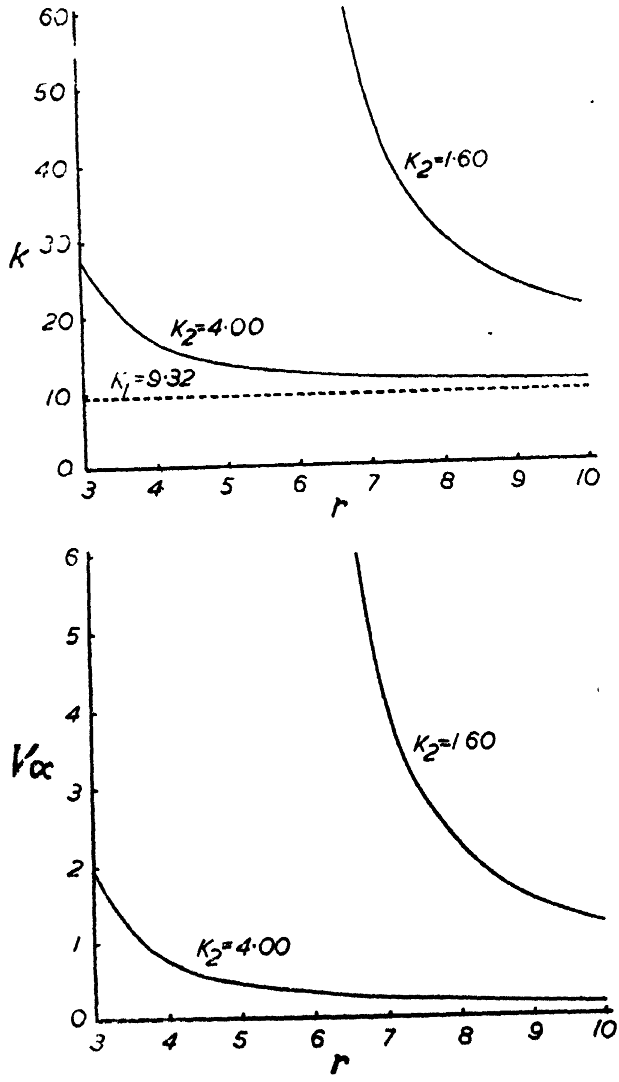


FIG. 15

The values of k (above) and V_α (below), assuming various values for r, when $K_1=9{\cdot}321$, and $K_2=1{\cdot}600$ and $4{\cdot}000$. Fuller details will be found in the text

Even the minimal value of k would, therefore, demand the linkage of at least five of the units. That this linkage escaped detection in the test of Section 17, merely serves to emphasize that, as has already been stated, the linkage test cannot be regarded as sensitive in the absence of replication adequate to provide a reliable estimate of error, such as was obtained in the barley experiment and to a lesser extent with the oats. Residual factor interaction could serve to obscure linkage in the only type of test possible with the *Petunia* data.

Now where a chromosome pair differs in a number of genes scattered along its length and all affecting the character in question, any chiasma which may form will, as it were, serve to distinguish two super-genes. The gross aggregate of all the genes within each of the two segments which have recombined at the chiasma will be transmitted as a unit. If two chiasmata form, three such super-genes will appear. The chromosome will thus appear to consist on the average of super-genes to a number greater by one than its mean chiasma frequency. The greatest number of effective factors, i.e. the greatest k which can be found, will thus be given by what Darlington (1939) has called the recombination index, viz. the haploid number of chromosomes plus the mean chiasma frequency. This would be somewhere between 17 and 20 in *Petunia*—a value in reasonable keeping with the estimate of k. The effective factor as we have been using it is in the general case therefore not an ultimate gene, but merely a segment of chromosome acting as a unit of inheritance and separated from other such units by an average recombination frequency of 50%.

Since the positions in which chiasmata form may vary from nucleus to nucleus even within the same individual, the super-genes which the chiasmata distinguish will not be of constant content. They will be variable even within a generation, and V_β will in consequence be high. The value of K_2 should therefore be very much lower than that of either k or even of K_1 where there are polygenes scattered all along the chromosomes. This expectation accords well with the results from the *Petunia* species cross.

Finally, since chiasmata vary in position, a further breakdown of the effective factors must occur in later generations. The total number of factors found in these later generations will generally be greater than the first estimate. The effective unit of inheritance is thus a unit only for one generation, and even within this period

it may well be a statistical rather than a physical unit. This cannot of course prevent us using the unit for our statistical purposes. It merely serves to emphasize that the 'gene' of biometrical genetics, unlike the gene of Mendelian genetics, is not an ultimate unit. It is more like the 'gene' of structural change (Darlington and Mather, 1947).

Our factors may be treated as units for the purposes of calculation. If the chiasmata were invariable in the way they separated the factors within the chromosome, the factors would in fact be constant units and could be treated as final genes. With variation in the points at which the factors are delimited by chiasma formation, the factor will itself be variable, but we may still regard it as unitary within a generation provided that we recognize the existence of variation in the effect of the unit. In doing so we are, as it were, replacing the factors which potentially merge into one another along the chromosome by a series of separate genetical centres of gravity, one for each factor. Each centre of gravity will have an average effect which reflects the mean genic content of the factor it represents, and a variance of effect which reflects the variation in genic content of that factor. The centres of gravity will normally be regarded as occurring every 50 recombinational units along the chromosome, because we would generally find it most useful to assume free segregation when estimating the number of factors. But in special cases where the linkage test has revealed an association between the factors in hereditary transmission, we may regard the centre of gravity as separated by less than 50% recombination for the purposes of our calculations. This course of action will be illustrated by reference to the barley example.

The model is, of course, artificial in the sense that it has a discontinuity for which there need be no strict physical or physiological counterpart in the chromosome. It can be set up only as an approximation for the purposes of calculation, and it cannot be used uncritically over a range of generations within which the factors may themselves be changing in average number and average effect as a consequence of variation in the position of chiasma formation. More experimental evidence will be needed before we can establish the range of validity of the model and see how it can be adjusted to meet the needs of any change which may occur in the effective factors.

21. PREDICTION IN THE PRESENCE OF LINKAGE
(in collaboration with Dr. U. Philip)

The prediction of selective advance, as developed for the oat experiment, depends on regarding K_2 as a reasonable estimate of k, and hence of the K_1 which would be expected from a cross between two lines with isodirectional distribution of + and − allelomorphs. We have just seen that when linkage is encountered K_2 may be sharply reduced by the variation in individual effect of the effective factors. Prediction, based on the value of K_2, is thereby made less efficient, or even completely vitiated in extreme cases.

Linkage also acts in another and more direct way to reduce the values of both K_1 and K_2. When two genes are unlinked they appear distinct in inheritance. When they are completely linked they act as one factor of effect (d_a+d_b) if in coupling or (d_a-d_b) if in repulsion. Intermediate linkages will give situations in which a number of factors intermediate between two and one appear to be acting, even though once a recombination has been achieved in the case of repulsion the selective advance will be that appropriate to two units.

Linkage can be seen at work in these ways in the barley experiment. The deviation of the parents from the mid-parent value is 13·67, and the value of D is 10389. Thus $K_1=\frac{13{\cdot}67^2}{10389}=0{\cdot}018$. This small value indicates the association of + and − allelomorphs in both parents as already noted in Section 18.

Low values of K_2 cannot, however, be due to this cause. Now the heritable portion of $\bar{V}_{F3}$ is 4314−1224=3090, and the harmonic mean size of the F_3's is 7·84. V_{VF3} is 21283108, from which a correction of $\frac{2\times4314^2}{7{\cdot}84-1}$ is subtracted, leaving 15841413. Thus

$K_2=\frac{3090^2}{15841413}=0{\cdot}603$. Linkage cannot in any case directly reduce the number of effective units below 1, and in the present instance it could not be expected to reduce the number below 2, since we have already seen that there is evidence of at least two linkage groups being involved (Section 18). Part of the reduction must, therefore, arise from the variation in expression of the individual effective factors.

This value of K_2 is obviously of little use for the purpose of predicting selective limits, except to tell us what we already know, that the concentration of allelomorphs is not complete in the

parents. We may, however, use another property of linkage for the purpose of prediction. In F_2, $D=S(d_a^2)\pm 2S[d_a d_b(1-2p)]$; and in F_3, $D=S(d_a^2)\pm 2S[d_a d_b(1-2p)^2]$, the sign depending on the phase of linkage between the individual pairs of genes. In general with preponderant coupling D_{F3} will then be less than D_{F2}, as observed in the barley, and the magnitude of the fall from F_2 to F_3 will depend on the relative magnitudes of d_a, d_b, &c., on the recombination frequencies, and on the number of genes involved. The fall will be maximal when all genes are both coupled and of equal action, and when adjacent genes are equally spaced along the genetical chromosome. We cannot know of course that our factors are either localized in distribution or invariable in genetic content like single genes. We may however regard them as represented by genetical centres of gravity spaced out along the chromosomes like the genes in terms of which the discussion has been carried on. The genetic weight at each centre will be variable, but the variation should do no more than make our estimates of the fall in D, calculated on the assumption of invariable genes, too high and the number of factors correspondingly too low. This underestimation will not be seriously misleading because even if single genes were involved we could aim only at a minimal estimate.

The precise recombination frequency giving maximal fall will vary with the number of factors, but it can clearly be calculated for any given number of them. One point must, however, be made clear before proceeding to the calculation, viz. that maximal must be taken as meaning maximal in relation to the value observed for D_{F2} or D_{F3}. Otherwise there is no standard of reference. We shall take D_{F2} as the basis for our determinations of maximal change.

The fall in D is $S(d_a^2)+2S[d_a d_b(1-2p)]-S(d_a^2)-2S[d_a d_b(1-2p)^2]$ and its ratio to D_{F2} is thus $\frac{2S[d_a d_b(1-2p-\overline{1-2p}^2)]}{S(d_a^2)+2S[d_a d_b(1-2p)]}$, which becomes $\frac{4d^2S[p(1-2p)]}{d^2[k+2S(1-2p)]}$ when k factors all having effect d are concerned. With $k=2$ this is $\frac{4p(1-2p)}{2+2(1-2p)}$. The maximal *fall ratio* is then 0·17 when p is 0·29. Assuming the absence of interference, and equal spacing of adjacent factors, the maximum fall ratio with $k=3$ is 0·25 when adjacent factors show $p=0{\cdot}27$ recombination. Similarly the ratio is 0·30 at $p=0{\cdot}26$ when $k=4$. Thus a fall of, say, 0·22 requires a minimum of 3 factors. A fall ratio of 0·28 would

similarly require at least 4 factors. With interference between chiasmata, both the maximum fall ratio and the value of p which gives it are lower than when it is absent (Table 30).

TABLE 30

Maximum Fall Ratios in D and H with k=2, 3 and 4 in Coupling and Reinforcement respectively

	k	p	Fall in $D\left(\frac{D_{F2}-D_{F3}}{D_{F2}}\right)$	$\frac{S(d^2)}{D_{F2}}$	Fall in $H\left(\frac{H_{F2}-H_{F3}}{H_{F2}}\right)$	$\frac{S(h^2)}{H_{F2}}$
	2	0·29	0·17	0·71	0 06	0·85
No Interference	3	0 27	0 25	0 58	0 09	0 77
	4	0 26	0 30	0 50	0 12	0·71
Complete Interference	3	0 20	0·22	0·52	0 11	0 67
	4	0 16	0 24	0 40	0 13	0 54

In the case of the barley D_{F2} is known, but D_{F3} cannot be separated from H_{F3} in the absence of F_4 data. There will, however, be a fall in H, similar to, though less than, that in D, if the h increments are preponderantly reinforcing (Fig. 10 and Table 30). If we therefore use $D+\frac{1}{2}H$ in place of D for calculating the fall, the result will be to slightly underestimate its magnitude. This only means a slightly lower value for k, a minimal estimate of which is being aimed at in any case. In F_2, $D+\frac{1}{2}H=10389+\frac{1}{2}(13169)=16974$. In F_3, $D+\frac{1}{2}H$ can be found by subtracting E_1 (as estimated when allowance is made for linkage) from $\bar{V}_{F3}$ and multiplying by 4. It is, therefore, $4(4314-1224)=12360$. The fall ratio is then $\frac{16974-12360}{16974}=\frac{4614}{16974}=0·27$. Three coupled factors will not quite suffice, since their maximum fall ratio is 0·25. Four will be enough, since their maximum fall ratio is 0·30.

We have already seen however that there must be at least two linked groups of genes, each with preponderant coupling, but balancing one another's actions almost exactly in the parents. Now two coupled groups each of k factors will give the same maximum fall ratio when segregating simultaneously that each does by itself. With unequal numbers of factors, the simultaneous fall will be intermediate in value between the individual ratios. Thus in the present case we must suppose, with two groups acting, that more than 6 factors are operating, with over 3 of them lying in each group. (Two in one group and four in the other gives no better a fit than three in each, the fall ratios being much alike in each case.) Eight factors, four in each group, would be ample to

explain the results. Seven factors, four in one group and three in the other, would also explain the fall, but might not account for the close balance of effects in the parents.

Now with coupling the value of D_{F2} exceeds $S(d^2)$ by an amount depending on the number of factors and their linkage relations. When $k=2$ and $p=0{\cdot}29$, the value giving the maximum fall ratio of 0·17, $D_{F2}-S(d^2)$ is 0·29 of D_{F2}. A factor of 0·71 must therefore be multiplied to D_{F2} in order to give $S(d^2)$. The corresponding factors for $k=3$ and $k=4$ under the conditions of maximum linkage fall are given in Table 30. Thus the linkage fall ratio enables us to estimate the minimum number of factors which must be involved, and also the correction necessary for reducing D_{F2} to $S(d_2)$.

In the barley case, if we take the underestimate of $k=6$, 3 in each group, then $S(d^2)$ is 0·58 of D_{F2}, i.e. 0·58×10389 or 6025. If we regard each group as comprising 4 factors, giving 8 in all, $S(d^2)$ is 0·50×10389 or 5195. Then in the former case $kS(d^2)=6\times6025=36150$ and in the latter $kS(d^2)=41560$. Since the minimal limit to selective change in either direction is given by $\sqrt{kS(d^2)}$, it will be $\sqrt{36150}$ or 190 with 6 factors, and 203 with 8. The mid-parent was 314, and so the minimal limits should be 124 and 504 if 6 factors are assumed, or 111 and 517 if 8 are assumed. Actually the means of the extreme F_3 families were 181 and 568, the former inside and the latter outside the limits calculated on both assumptions. We have, however, already seen that the scale was not fully adequate (Section 18) and its distortion is such that the expected upper limit would be exceeded before the expected lower limit was obtained. The difference between the extremes observed in F_3 is 568−181 or 387, which lies between 2×190 and 2×203, the differences between the limits expected on the two assumptions. Thus there is no ground for expecting that much selective advance could be made on the extremes observed in F_3, though some progress might be made if the units of inheritance were either not so constituted or not so related as to give the maximum possible fall ratio. Since, however, the linkages are preponderantly in coupling, 100 F_3's would be very likely to contain families reaching the limits. Everything points, in this cross, to the F_3 families having already reached the limits of selective progress which might be envisaged as easily obtainable.

A further use may be made of the estimate of the number of factors in the present case. The F_1 with a mean of 400 showed heterosis. H_{F2} was found to be 13169. This value will, of course, exceed $S(h^2)$ by the item $2S[h_a h_b(1-2p)^2]$. The value of $S(h^2)$ will,

therefore, be less than H_{F2}, though not so small a part of it as $S(d^2)$ is of D_{F2}. With the values of p found in calculating the maximum fall ratios of D (assuming, of course, complete reinforcement of the h increments, in the same way as complete coupling of the factors was assumed when considering the d increments), the values of $\frac{S(h^2)}{H_{F2}}$ for $k=2$, 3 and 4 are found to be as shown in Table 30.

If we take $k=6$, 3 in each group, $S(h^2)$ is 0·77 of H_{F2}, i.e. 0·77×13169 or 10140. With $k=8$, $S(h^2)$ is 0·71 of H_{F2} or 9350. Then $kS(h^2)$ is 60840 in the former and 74800 in the latter case. The degree of heterosis to be expected if all the factors were reinforcing will thus be $\sqrt{60840}$ or 247, with $k=6$; and 274 with $k=8$. The heterosis observed was 400−314 or 86. Thus, even when the large error of estimation of H_{F2} is taken into account, the factors cannot be regarded as all reinforcing one another. New inbred lines could be extracted from the cross, which would give greater heterosis through the fixation of the + allelomorphs of these factors whose h increments were in opposition to the heterosis.

Such a calculation has little value in barley where homozygous varieties are used in practice; but it might well be of use in certain crops in deciding the value of inbred lines for hybrid seed production and assessing the prospects of their improvement.

22. THE SPEED OF ADVANCE UNDER SELECTION

Not only will the minimal limits of selective advance be determined by the number of units of inheritance which contribute to D and H, but the speed of selective advance must clearly also depend on this number. Where only one gene is segregating, $\frac{1}{4}$ of the F_2 individuals will be homozygous for each allelomorph. In the absence of obscuring agents, the selection of any number of individuals up to 25% at either end of the scale will lead to immediate fixation of the homozygous type in the next generation. All the progress possible will be achieved immediately.

With two genes, segregating independently, 6·25% of the F_2 individuals will be homozygous for each of the extreme genotypes. Thus the chance of recovering an extreme genotype from an F_2 of any given size is reduced. Furthermore, the progress made by selecting, say, 10% of the individuals at one end of the scale in F_2 cannot be complete even in the absence of obscuring agents.

Some genetically less extreme types must also be taken for breeding.

Where four genes are segregating only 1 individual in 256, i.e. 0·39%, will be homozygous for each of the extreme genotypes. Not merely is the chance of immediate fixation under selection very small, it is even unlikely that any extreme homozygote will be picked up in an F_2 of small or medium size. The group selected for further breeding must, therefore, include individuals of various genotypes showing various degrees of heterozygosity in proportions depending, among other things, on the magnitudes of the h increments. Progress will be incomplete, and only as the extreme homozygotes come to form an increasing proportion of the segregates in later selected generations will the full advance under selection become realized.

The efficacy of selection in such a case must also be reduced by the effects of non-heritable agents, which make the phenotypic expression of the various genotypes overlap and so reduce the chance of effective separation of the genotypes in selection. The greater the non-heritable variation, therefore, the less effective the selection. The variation in proportion of the different genotypes in F_2 consequent on sampling error may also reduce the efficacy of selection by reducing the frequencies of the more extreme and desired types. The efficacy of selection might, however, also be increased by a corresponding chance excess of these types in F_2.

Thus the speed of selective progress will depend on

(*a*) the rigour of selection (i.e. proportion of F_2 chosen for breeding);
(*b*) the number of genes, as organized into effective factors in the way we have discussed;
(*c*) the variation in magnitude of action of the genes or factors (variation in magnitude of d increments);
(*d*) their dominance relations (magnitudes of h increments);
(*e*) their linkage relations;
(*f*) the size of the contribution of non-heritable agents to the F_2 variation;
(*g*) the sampling variances of the genotype frequencies.

Some of these agents can be assessed in the ways already discussed. On others information will be less certain or even absent. The practical problem is to decide how much can be foreseen about the speed of progress from the statistics we have learned to calculate. This problem has been tackled by Panse (1940*a* and *b*), who

has developed a method of analysis and has shown how it works out in one set of cases.

The character Panse considered was staple-length in three cotton (*Gossypium arboreum*) crosses. The F_2 families were grown in one year and F_3 families in the next. It is impossible from Panse's data to undertake analyses of the kind necessary to separate D, H and E in his crosses. Panse himself (1940*a*), however, estimated what he terms the genetic variance * of two of the crosses. This was found by the regression of F_3 mean on F_2 parent value, i.e. as $\frac{\frac{1}{2}D+\frac{1}{8}H}{\frac{1}{2}D+\frac{1}{4}H+E}$. Values of 1·543 and 1·516 were obtained in his two cases, leaving residual variances (which he regards as non-heritable, though these, like the so-called genetic variances, will contain items depending on H) of 1·472 and 1·697 respectively. For simplicity Panse approximated by setting the total F_2 variance at 3, of which 1·5 is supposedly genetic and 1·5 not genetic, in each case.

The number of effective factors was estimated by K_2 for each cross, the one giving $K_2=1·64$ and the other $K_2=2·77$. Panse chose to take $K_2=3$ as the basis of his further consideration. In order to consider the effects of both dominance and unequal action of the genes involved he set up five genetical models. In three of them only three genes were involved. In the first the genes were equal in action and without dominance. In the second, two of the three genes were equal in action but showed complete dominance ($h=d$) in opposite directions, the third gene showing no dominance but contributing equally to the variance with the other two, i.e. $\frac{1}{2}d_c^2=\frac{1}{2}d_a^2+\frac{1}{4}h_a^2=\frac{1}{2}d_b^2+\frac{1}{4}h_b^2$. The third model also had two genes with complete dominance in opposite directions and one without dominance but it put $d_a=d_b=d_c$, so that the genes contributed unequally to the variance. The other two models involved an infinite number of genes, in the one case showing no dominance and with the values of d descending in geometric progression, and in the other case falling into two series of equal action, each with complete dominance but in opposite directions, the values of d descend-

* Panse's use of this term appears not to be consistent. In 1940*b* he follows Fisher (1930) in regarding as the genetic variance that which in the present notation would be described as the D (as opposed to H) portion of the variance. The method of estimating the genetic variance of F_2 used in 1940*a* must, however, lead to an estimate which will be affected by the magnitude of H. Finally, in setting up his genetic models, Panse calculates the contribution each unit makes to the genetic variance of F_2 as $\frac{1}{2}d^2+\frac{1}{4}h^2$.

ing geometrically within the series. Linkage was supposed to be absent from all the models, and the average contributions of the genes were adjusted to give $K_2=3$.

Taking these five models, the effect was considered of selecting the top 10% of the F_2 plants for further breeding by self-pollination. The mathematical method used for this purpose is over-elaborate for presentation here: it will be found in detail in Panse (1940*b*). Briefly, however, it consists of setting down a moment generating function for the simultaneous distribution of the F_2 parent values, the F_3 means and the heritable variances of the F_3's. On expanding, quartic regression equations are found relating the F_3 statistics to the F_2 parent values. These equations are then integrated between limits appropriate to the method of selection. The results of the mathematical operations are shown in Table 31, but with the scale changed from that used by Panse, so as to put the heritable portion of F_2 variance $({}_H V_{F2})$ equal to 1. This scalar change should enable the table to be used more easily with other genetical data, since if ${}_H V_{F2}=a$, the various columns of the table can be multiplied by the factors shown in its bottom row to give the values appropriate to the case.

As would be expected, the selective advances and the F_3 variances vary with the particular genetical model used; but the variation is surprisingly small. The advance achieved is at least 36% and at most 51% of the maximum possible. The different variances show an even smaller relative range. When the errors of estimation of the F_3 quantities are borne in mind, it is clear that the variation between models is not likely to lead to serious misjudgement. The results of selection can therefore be forecast with useful accuracy a generation or so ahead by this means.

In regard to the advance achieved expressed as a ratio of the maximum advance possible (and this ratio is the most important feature from the point of view of practical forecasting), the chief difference in the table is that between the three models assuming three units on the one hand, and the two assuming an infinite number on the other. The first group shows about $\frac{1}{2}$ the possible advance as achieved, the other just over $\frac{1}{3}$ of it. Since the units are assumed to be unlinked an infinite number is in practice an impossibility, so that the lower limit of advance achievable in F_3 must actually lie somewhat above the percentage shown for the two models with infinite series.

Panse developed his models in terms of genes. We have seen, however, that there can be no certainty that the units of segrega-

TABLE 31

Effects of raising F_3's from upper 10% of F_2 whose mean is zero, with Heritable Variance=Non-heritable Variance=1 in F_2, and K_2=3. (Panse's models)

Model	Limit of selective advance	Advance achieved in F_3	F_3 advance as proportion of that possible	$_H\bar{V}_{F3}$	$_HV_{F3}$	V_{VF3}
I No dominance Geometric series	3·415	1·235	36%	0·397	0 496	0·071
II No dominance 3 equal units	2·449	1·235	50%	0·399	0·499	0·070
III Balanced dominance 2 geometric series	2·642	1 034	39%	0·428	0·446	0·065
IV Balanced dominance 3 genes of equal variance	2·150	1·100	51%	0 419	0·462	0·068
V Balanced dominance 3 genes of equal action	2·121	1 084	51%	0 421	0·465	0·068
Where $_HV_{F2}=a$ multiply by	$\sqrt{a}$	$\sqrt{a}$	1	a	a	a^2

tion with which we are concerned will in fact consist of ultimate genes. Rather we must consider the possibility of groups of genes appearing as our factors. The surprisingly small effects of dominance and number of genes on the rate of advance under selection encourage us to believe that Panse's results will still apply broadly to systems of such factors, recombining freely with one another through lying in different chromosomes or through being separated by chiasmata if within the chromosome, even though the separation will not be into uniform factors in the latter case. In so applying Panse's results we are of course once again making the assumption that the full effect of each factor is concentrated at its genetical centre of gravity, which is recombining freely with other such centres. This assumption is artificial, but provided its limitations are recognized, it enables us to see Panse's calculations in their application to a wider field of experimental situations.

Though having no observations for illustrative purposes, Panse considered the use of his models for the purpose of detecting dominance and variation in magnitude of action of the units of inheritance by comparison of the various F_3 quantities calculated from observed results with those expected on the different assumptions. This application of Panse's method will serve to supplement the consideration of these questions by the analyses developed earlier. The chief use of the method must be, however, in predicting the relative advances to be expected in F_3—a use whose value it is difficult to over-rate. The case in question, where ${}_HV_{F2}={}_EV_{F2}$, $K_2=3$ and 10% selection, is of course only one of the many which might be encountered; but it is possible by the use of Panse's method to tabulate the advances expected where other selective rigours are used, where K_2 takes other values, and where ${}_HV_{F2}$ is differently related to ${}_EV_{F2}$. Such tables would be a powerful aid to the geneticist and breeder in the use of his biometrical data.

Panse did not take into account the effects of linkage in his calculations, but its consequences are not difficult to see in general terms. Linkage in either phase reduces the value of K_2. The speed of advance under selection, relative to the limiting advance as immediately foreseeable from the data, must therefore be greater than it would be in the absence of linkage. There is, however, an all-important qualification in the case of repulsion linkage. The above statement is true in so far as the limits of advance are calculated on the basis of the existing linked combinations; but crossing-over will give new combinations, which must widen the ultimate limits of selective advance. Thus while the speed of

advance relative to the limits set by the existing combinations may be greater than in the absence of linkage, relative to the limits set by the new combinations which follow recombination, the speed of advance will generally be slower than in the case of no linkage. This is the reason for the favouring by natural selection of balanced polygenic combinations with repulsion linkages (Mather, 1941). With coupling linkages, on the other hand, recombination can act only to narrow the ultimate limits of advance under selection. The sole effect of linkage in coupling is consequently to lead to a greater speed of advance towards these limits, as we have seen in the case of ear conformation in barley discussed in the previous section.

The concept of the number of effective factors is thus one which in suitable circumstances allows us to estimate the minimal limits of selective advance, minimal in the sense that K_2 may be under-estimated as a result either of linkage, especially in the repulsion phase, or of variation in the magnitude of effect of the factors. It also allows us to learn something of the rate at which we may achieve these advances when using selection of a given rigour. The effective factor is thus the basis in biometrical genetics upon which our predictions depend. It is a unit whose properties we shall discuss further in the next chapter.

CHAPTER 7

RESULTS AND CONCEPTS

23. EXPERIMENTS IN BIOMETRICAL GENETICS

MANY EXPERIMENTS have been carried out at various times on the inheritance of quantitative differences, but few of these have been of a kind which can lead to a partition of the variation into its D, H and E components. Even fewer have been designed in such a way that the tests are sensitive and the analysis reliable. Fifteen experiments, including either F_2 or biparental families have been analysed, with there sults shown in Table 32(*a*). Of these, four are experiments, three hitherto unpublished, carried out at the John Innes Horticultural Institution, one is Quisenberry's (1926) experiment with oats, seven are from Emerson and East's (1913) investigations on maize, and three are from Culbertson's (1942) experiments with sugar-beet. Though a number of other accounts in the literature show clearly that data must have been available of the kind needed, they are not presented in such a way as to permit analysis.

Even of the fifteen experiments analysed, only one can be regarded as fully adequate in design and magnitude, viz. that on ear conformation in barley conducted in collaboration with Dr. U. Philip. The *Petunia*, *Antirrhinum* and *Drosophila* experiments were made with all the necessary precautions of randomization and the inclusions of parental and F_1 lines to provide measures of E_1 and E_2; but even in the largest of them, that with *Drosophila*, the biparental families numbered only 31, while in *Petunia* and *Antirrhinum* only 19 and 8 F_3's were grown respectively. Furthermore in each case at least one parental line was not inbred and therefore could not be regarded as genetically uniform. This latter fault of the three experiments should not, however, be regarded as unduly serious for two reasons. In each case the genetical differences between the parental strains was much greater than those within them; and in so far as it is desired to investigate the genetical differences between strains, differences within them may be regarded as extraneous sources of variation, like the non-heritable differences with which they are confounded. A more serious deficiency lies in the design of these three experiments, for no provision was made for replication. In consequence no basic estimate of

TABLE 32 (a)

Species	Author	n	No. of F_2's or BIP's	D	H	E_1	Linkage	K_1	K_2	k or other K	Character
Avena sativa	Quisenberry (1926)	21	150	1·3211	1·0694	0·3653	A	4 501	5·127	—	Grain length
Petunia axillaris × *violacea*	Mather (unpublished)	7	19	0·7258	0·0361	0·1244	C	9 321	1·600	11–30	Corolla length
Drosophila melanogaster	Mather (1941)	4	31	1·5263	−2·9323	6·5709	R	—	—	—	Chaetae number
Hordeum sativum	Mather and Philip (unpublished)	7	100	10388·8	13169·0	1224·0	C	0·018	0 603	6–8	Ear conformation
Zea mays	Emerson and East (1913), Table 8	10	8	2·7887	−5·1910	1·8261	A	1 842	0 006	—	Row number
	10		8	6·3897	−10·5702	1·6764	A	0 312	0 018	—	Row number
	12		16	8·4143	−2·7043	- 3·1752	A	0 925	1 094	—	Row number
	19		16	36·6585	−1·5450	8·1399	C	5 979	—	—	Diameter of ear
	24		7	427·01	−495·82	107·17	A	3 761	4 105	—	Breadth of seed
	29		16	28·9012	−31·9361	2·7808	A	2 084	5 447	—	Height of plant
	33		16	−194·4866	1542·8651	103·0197	A	(−0 663)	8·708	—	Internode length
Antirrhinum majus × *glutinosum*	Mather (unpublished)	16	8	101·5113	52·5995	17·5360	R	0 610	2 634	—	Number of flowers
Beta vulgaris	Culbertson (1942), Table 6	18	30	0·8379	−1·1074	0·9326	A	0 511	0·002	—	Sugar %
	7		24	0·6046	2·0139	0·9799	A	5 734	0 198	—	do.
	8		23	3·0043	−5·2218	0·9572	A	2 107	0 224	—	do.

In the Linkage column A=absent
C=preponderantly coupling
R=preponderantly repulsion
n=haploid number of chromosomes.

TABLE 32 (*b*)

Species	Author	n	$\frac{1}{2}D+\frac{1}{4}H$	E	K_1	
Gossypium species crosses	Leak (1911)	13	24·69	47·30	13·5	Flowering time
Oryza sativa	Hoshino (1915)	12	47·76	2·87	3·2	do.
Triticum sp.	Thompson (1918)	21	6·09	2·68	0·6	do.
			4·51	1·49	1·0	do.
			7·43	2·33	1·3	do.
			17·66	2·03	0·6	do.
			5·72	2·06	3·4	do.
			10·63	2·07	2·5	do.
			26·72	2·09	1·1	do.
			13·95	2·13	2·3	do.
			8·03	2·31	4·2	do.
			27·68	2·26	2·0	do.
Triticum sp.	Freeman (1919)	14	6·37	9·63	3·8	do.
			12·94	2·90	1·4	do.
Triticum sp.	Florell (1924)	21	21·91	6·09	2·1	do.
Pisum sativum	Rasmusson (1935)	7	2·88	5·10	0·0	do.
			−5·61	13·04	—	do.
			−1·09	8·83	—	do.
			14·10	5·82	1·0	do.
			13·96	4·60	0·5	do.
			3·20	10·06	3·2	do.
Solidago sempervirens	Goodwin (1944)	9 or 18	—	—	5·2	Floral initiation
			—	—	8·7	
			—	—	2·5	Floral initiation to anthesis
			—	—	0·5	
			—	—	5·8	Anthesis
			—	—	6·4	

error was available. Without such an estimate of error the test for linkage must be insensitive, as we have seen in regard to the *Petunia* results (Section 17). Furthermore, no test is possible of the genic interaction remaining after scaling, such as could be made on the oats (Section 17) and the barley (Section 18).

Quisenberry's experiment with oats is satisfactory in that it was large, was made in duplicate, and included true-breeding parents. It could have been improved for our purpose by growing the F_2 in a number of groups each of the size of one F_3 family, in the way used for the parental lines, and also by including parents, F_2 and F_3's in a single randomized design. Nevertheless, as has been seen,

the data from this experiment have proved both informative and consistent to a remarkable extent.

The seven maize examples were taken from a larger number of experiments undertaken by Emerson and East. The remainder of these were excluded on either or both of two grounds; (*a*) that the data were incomplete or insufficient in regard to the number of F_3 families used, and (*b*) that the F_2 plants listed as parents of the F_3 families showed evidence of not constituting a random sample of the F_2 families from which they were taken. Even out of the seven experiments analysed, one had so low a number of F_3's as seven, and none had more than sixteen. The parental lines were not inbred, no consistent replication was practised, and randomized designs were, of course, not used. Furthermore, the various families of one experiment were often grown in different years and were of very diverse sizes. Much the same remarks apply to the three sugar-beet crosses which complete the table.

In spite of their various deficiencies in the experimental materials and methods, the data given in Table 32 still have their value for comparative purposes. Indeed, their very deficiencies are instructive since they both bring home to us the consequences of inadequate experimentation and show us how better experiments may be designed.

The second part of the Table (32*b*) includes 27 sets of data taken from Goodwin (1944). These all relate to the inheritance of flowering time and are all derived from observations on parents, F_1 and F_2. They permit therefore neither the separation of D and H nor the test of linkage, though the non-heritable and total heritable parts of the variability in F_2 can be estimated separately. In the same way K_1 can be estimated using $D+\frac{1}{2}H$ in place of D, but K_2 cannot be estimated at all.

Variability, whether E, D, H or the combination of D and H in the form of the total heritable portion, is measured as a variance, i.e. as a quadratic quantity. All the portions into which the variability is partitioned must therefore have positive values. A number of negative quantities appear in the table, but in no case where a test has been possible does the negative value differ significantly from zero, or even, in most cases, from a wide range of positive values. These negative quantities may therefore be fairly ascribed to sampling error, which is of course large in the less well-designed experiments. It is instructive to note that, as would be expected from the various c matrices used, the most efficient partition of the variability is that between the non-heritable portion on the

one hand and the combined heritable portion on the other. No negative value has been found for E, and $\frac{1}{2}D+\frac{1}{4}H$ is positive in all cases except for two taken from Goodwin's compilation. The reason for this easy separation into heritable and non-heritable is clearly the provision of a direct estimate of E measured by the variation of true-breeding parents and their F_1's.

No such direct estimates are possible of either D or H from F_3 progenies, though $W_{F2/BIP}$ is a direct measure of $\frac{1}{4}D$. The separation of D and H will generally depend on comparisons of quantities such as $\frac{1}{2}D+\frac{1}{4}H$, $\frac{1}{4}D+\frac{1}{8}H$, $\frac{1}{2}D+\frac{1}{8}H$, and as the c matrix shows, the values so obtained for D and H will have a high negative correlation and will be subject to higher sampling errors than is E. In particular H, when estimated by the use of F_3's or biparental progenies, has a very high sampling variance, ranging from 75 to 130 times as large as that of E, and even from 6 to 10 times as large as that of D. The consequences of these relations are brought out clearly in Table 32. The two sizeable experiments and the only two in which adequate replication was practised, on oats and barley, give positive values for both D and H, though only in the latter is H significantly greater than 0. The remainder were smaller non-replicated experiments, and the estimate of H very commonly appears with a negative sign. Owing to its lower sampling error the estimate of D is negative only once.

In these cases, whether it be D or H which has a negative estimate, the other quantity appears with a correspondingly large, and correspondingly spurious, positive value. Thus, in the *Drosophila* experiment for example, D is found as 1·5263 and H as −2·9323, giving as the heritable component of the F_2 variation $\frac{1}{2}D+\frac{1}{4}H=0{\cdot}0301$. While this latter may well be an overlow estimate, the values found for D and H are clearly untrustworthy. The test of linkage is not thereby jeopardized, because it rests on comparisons not of D with D, or H with H, in the variances of F_2 and later generations, but on comparisons of quantities derived jointly from D and H such as $\frac{1}{2}D+\frac{1}{4}H$ in F_2. The negatively correlated departures of the estimates of D and H from the true values will largely cancel out in this test.

When seeking to estimate the limits and rates of advance under selection on the other hand, D must be separated from H. Such spuriously large values as that found in the *Drosophila* experiment must then prove to be most misleading. In such a case it may well prove more reliable to substitute an adjusted value of D, found as $\frac{4}{3}(D+\frac{1}{4}H)$, if dominance is assumed, or as $D+\frac{1}{2}H$, if no dominance

is assumed. In the cases of the only two large experiments, those on oats and barley, the use of these formulae, particularly $\frac{3}{4}(D+\frac{1}{2}H)$ would lead to predictions of selective advance very like those made with the aid of the direct estimate of D. The same is also true of the *Petunia* and *Antirrhinum* experiments, even though they are much smaller. In these cases little is to be gained by the adjustment; but in the remainder the use of the adjusted estimate has much to commend it. This last group of experiments was, of course, expected to give somewhat unreliable results, for reasons already set out. Thus until a greater body of more adequately designed and conducted trials is available for consideration, it must remain impossible to say how far adjustment of D will be of general value.

Though it will be impossible to avoid the negative correlation between the estimates of D and H, the relative distortions to which estimates of the two are subject by reason of sampling error may be more nearly equalized if backcross families are included in the experiment. Even biparental progenies have the advantage of F_3's in this respect, as can be seen by comparison of the c matrices developed for the *Drosophila* and *Petunia* experiments in Section 13 (Tables 14 and 16). In backcross progenies D and H contribute equally to the various statistics which may be calculated from the data. Consequently the sampling errors of D and H will be more nearly equal in an experiment which includes backcrosses. Additional advantage is secured where the covariances are available of non-recurrent second backcrosses with the first backcross parents, because these statistics depend on $D-H$ for their values. Their inclusions must therefore reduce the negative correlation between the estimates of D and H, as well as help to equalize the sampling variances.

The use of any or all of backcross, second backcross and biparental progenies to supplement F_2 and F_3's is of further value in a different way. The test of linkage can be made more precise merely by replication of an experiment including only F_2 and F_3's, because in this way an estimate of error, more informative because based on a larger number of degrees of freedom, is made available for comparison with the linkage mean square. In the same way the residual genic interaction, against which the linkage component must be tested in the absence of replication, may itself be tested for significance when replication is practised (Section 17). The mean squares for linkage and residual interaction will however themselves each be based on only one degree of freedom. Where either backcrosses of the various kinds, or

biparental progenies, or both are also included in the experiment the mean squares for linkage and residual interaction may themselves be derived from a greater number of degrees of freedom. The tests of their significance can then be made with an increase in precision not achievable by mere increase of replication. Furthermore, the inclusion of a greater variety of types of family, by giving a wider inductive basis for these tests, should also render possible a more searching inquiry into the organization of any linkage group or interacting system that may be brought to light.

Some of the properties of linkage groups have already been discussed when analysing the linkage fall shown by D and H in the barley (Section 21). It is also clear from the suggestions on the one hand of coupling linkage of the genes controlling corolla length in the cross between the two *Petunia* species, which were chosen for their diversity in this character, and on the other hand of repulsion linkages in *Antirrhinum* and *Drosophila* where the parents, in the former case also different species, were not chosen for diversity in the character under examination, that the predominant phase of linkage found in any cross will be related to the way in which the parents are selected. A more searching experimental inquiry into the biometrical consequences of linkage is obviously needed, and the appropriate selection of parental lines for the work should do much to ensure its success.

The estimation of the number of effective genes also makes its own demands on the experimental design. K_1 is found as the ratio of the mean parental difference, halved and squared, to D. Anything which improves the estimates of $\bar{P}_1-\bar{P}_2$ and D must correspondingly increase the precision of the estimate of K_1. In particular, randomization is necessary to ensure the absence of bias from the measure of $\bar{P}_1-\bar{P}_2$, and replication will serve to reduce the error variance of the estimate of this quantity. The measurement of D has already been considered at some length, and it is only necessary to add that in this case too an adjusted estimate may well be more useful than the direct estimate of D. In short, an experiment which secures a good partition of the components of variation will generally provide an equally good estimate of K_1.

A new problem arises, however, when we turn to K_2. This is estimated as the ratio of the square of the mean heritable variance of F_3 to the variance of this variance. The estimation of the mean heritable variance itself brings in no new considerations, for we find it as $\frac{1}{4}D+\frac{1}{8}H$. As we have already seen, this quantity will be

estimated with greater precision than either D or H alone. The variance of the F_3 variance is, however, found directly from the F_3 variances. The greater the number of F_3 families, the better will be the estimate of V_{VF3}. Estimates from the oat and barley experiments, with 150 and 100 F_3's respectively, should be precise. Even those from the experiments in Table 32*a* with 16–19 F_3's should be reasonably reliable; but little precision can be expected where only 7 or 8 F_3's were grown. The variance of F_3 variances includes, however, a part which is merely a consequence of sampling error, and a correction $\frac{2}{n-1}\bar{V}^2_{F3}$ is deducted to adjust for this inflation. The accuracy of the estimate of $\bar{V}_{F3}$ will again depend on the number of F_3 families; but the magnitude of the correction depends also on the number of individuals in each F_3 family, or on their harmonic mean where the numbers differ. Large F_3 families are therefore needed if this correction is to be kept small. Thus the reliability of the estimate of V_{VF3}, and with it that of K_2, depends both on the number of F_3 families and on the number of individuals in each family.

The deficiencies of a number of the experiments in Table 32 are clearly shown by the negative values which they yield for D and H, particularly H. No such simple criterion is available in regard to K_1 and K_2. Indeed, on theoretical grounds, K_1 may take any of a great range of values. The barley data give K_1=0·018, while the oats give K_1=4·501, and both these are expected to be statistically reliable estimates. A negative, and therefore impossible, value of K_1 is recorded only once, and it was then due to a negative estimate of D.

K_2 is a little more helpful. Theoretically it may be larger or smaller than K_1, but if smaller, the difference should seldom be very great unless the effective factors are themselves highly variable in effect. With the Petunias K_1 is nearly six times the size of K_2. This either requires a very high variation of the factors or it suggests that although the estimates are not biased, they are of low precision as a result of the smallness of the experiment. The estimate of K_2 must also be suspect where it is less than 1. In the barley experiment this happens as a consequence partly of the linkage found to exist between the units and partly of variation in effect of the factors; but in some of the maize experiments it seems more likely to be due to the smallness and poor design of the experiments.

Exploratory experiments using 100 F_3 families each of 100 plants

have been suggested by Fisher, Immer and Tedin (1932). A trial including 10,000 plants of the F_3 generation alone is very large and has yet to be attempted. The oat and barley experiments probably came nearest to it, with between a third and a half of that number. In many cases experiments of such a size will be impossible; but until the questions just raised have been explored further, both theoretically and experimentally, it will be impossible to say how best to divide the facilities which may be available for an experiment between number and size of F_3 families. One consideration must, however, favour more families of lesser size, at least in plants, provided this size is not reduced below, say, 10 or 15 individuals. In so far as replication is practised; it is possible to undertake the replicated parts of the experiment a few at a time, provided that in the first place sufficient seed is produced of each family to permit the necessary successive sowing. The number of plants in each F_3 can in this way be increased step by step, to any extent that is shown to be necessary by the accumulating data themselves.

It should also be observed that neither biparental progenies nor backcrosses will add to the precision of the estimate of K_2, for this statistic is derivable solely from F_3 data. Indeed, biparental progenies are valueless for estimating the number of effective units, though second backcrosses can be used to provide their own estimate, which we may call K_3, of k. The most desirable balance between F_3's, biparental progenies and backcrosses therefore poses its own problems, which will again need experimental investigation. No data exist at present from which either the calculation of K_3 can be attempted, or the relative merits of K_2 and K_3 assessed.

Clearly the best design for experiments of the kind under discussion cannot yet be specified; and indeed it must always remain to some extent contingent on the nature of the material. With animals F_3's are impossible, and reliance must be placed on biparental progenies and on backcrosses. With cereals, on the other hand, the natural self-pollination mechanism makes F_3's easy to obtain, while crossing is sufficiently troublesome by comparison to render backcrosses and biparental progenies much less profitable for use. In other plants, e.g. maize, there is little to choose between F_3's and crossed families in regard to ease of raising, so that a fully balanced design can be secured without great difficulty. The species will in fact itself dictate the type of family on which the experiment must largely be based.

There are, however, certain general principles of design which

will hold good whatever the limitations imposed by the species. In the first place the inclusion in the experiment of parents or F_1 is necessary in order to provide a direct estimate of the E portion

P_2	F_3-16	F_3-24	F_3-18
F_3-3	P_1	F_3-1	F_3-25
F_3-9	F_3-22	F_3-21	F_2
F_1	F_3-20	F_3-19	F_2
F_3-13	F_3-12	F_3-4	P_2
P_2	F_3-14	F_2	F_1
F_2	P_1	F_3-11	F_3-15
F_2	F_3-2	F_3-6	F_3-7
F_2	F_3-5	F_3-17	F_1
F_3-23	P_1	F_3-8	F_3-10

FIG. 16

Design of a block including 40 randomly arranged plots. Of these 40 units, 3 are given to each parental strain (P_1 and P_2), 3 tote F_1, 6 tho the F_2, and 1 to each of the 25 F_3's (1–25). E_2 is estimated from the pool of squared deviations of the three plot means of P_1, the three plot means of P_2, and the three plot means of F_1 from their respective block means. E_1 is estimated from the pool of squared deviations of the individuals in the P_1, P_2 and F_1 plots from their respective plot means. V_{F2} is estimated from the pool of squared deviations of the F_2 individuals from their respective plot means, so that its non-heritable component is E_1

of variation. Furthermore, each parent or F_1 should be included several times within each replicate of the experiment so as to give a direct estimate of E_2 as well as E_1, i.e. of the non-heritable variation between family means as well as that between individuals. In mammals it may also be necessary to make provision for estimates of special non-heritable effects arising from the maternal influence in prenatal life (Lush, 1943; Chapman, 1946).

Secondly, the families should be randomized within the experiment to permit the effects of environment to be disentangled from the heritable variation. Where it is desired that one family, e.g. a parent or the F_2, should contain more individuals than another, e.g. an F_3, the larger one should be broken up into parts, each the size of the smaller, and the various parts randomized separately. The experiment should thus consist of a number of equal-sized units in random arrangement, the increase in size of particular families being achieved by allocating to them an appropriate number of these randomized units. The variance between means of units of the same parent or F_1 then gives a direct estimate of E_2, the non-heritable variance of $V_{\bar{F3}}$ or $V_{\overline{BIP}}$ (see Fig. 16).

Thirdly, replication is necessary to provide adequate tests of linkage and residual genic interaction.

Fourthly, as many different types of segregating families as convenient, particularly F_3's, bi parental progenies and second backcrosses, should be used in order to facilitate the separation of D and H, and to provide as wide a basis as possible for the detection and interpretation of linkage and residual interaction.

Finally, either F_3's or second backcrosses should be used to permit the calculation of K_2 or K_3. An appropriate balance should be aimed at between the number and the size of these families, to give as precise values for these quantities as facilities allow. With these precautions, the experiment may still fall short of the ideal, even for the size which available facilities permit; but it will certainly be both trustworthy and informative within the limits set by its design.

24. DOMINANCE AND INTERACTION

The study of continuous variation demands a combination of genetical theory with biometrical analysis. The hereditary determinants of continuous variation are carried on the chromosomes. Like other genes they segregate and they show linkage. If therefore we are to understand the variation which they control we must seek to measure their segregation and their linkage; and in doing

so we must take account of their physiological relations in the form of dominance and interaction.

Now the partition of variation into its components would be impossible without the foundation of Mendelian genetics upon which to build. We could no more devise the means of partition than we could interpret its results, if we were not familiar with the phenomena of segregation and linkage, of dominance and interaction. But our biometrical methods do not, and indeed cannot, lead to measures of their effects exactly like those measures which we use in Mendelian genetics. In place of the segregation ratios of genes with individually identifiable effects, we observe their pooled effects in the form of D. In the same way the pooled dominance is measured by H, and all the linkages act together in changing the values of D and H over the generations. The genes are always dealt with in the aggregate and we therefore learn nothing of their individual properties. The first results of analysis show us in fact only the combined effects of all the genes, though if we can find the number of effective factors it is of course possible to determine average effects and sometimes even the variances of the distribution of effects.

Thus biometrical analyses yield results which are in a sense complementary to those of Mendelian analysis. Where the genes can be followed individually we can discover their individual properties, but we cannot be sure without further tests that all the genes affecting the variation have in fact been traced. Biometrically we cover the whole of the variation, but we cannot measure individual properties. We are examining the same basic genetical phenomena by the two methods, but we are examining them in different ways. Not only will our measures of their effects be different, but properties of the genes which are finally distinguishable in simple Mendelian analysis may not be so when we are analysing variation biometrically.

There exists an ambiguity of this kind between the effects of dominance and genic interaction in biometrical analysis. Dominance itself may be regarded as a form of interaction, or non-additiveness in effect, between allelomorphs of the same gene. It was of course observed, and its consequences for genetical analysis were understood, by Mendel. In his peas the offspring of a cross so closely resembled one of the parents in each character that the effect of the other parent 'either escapes observation completely or cannot be detected with certainty'. A single dose of the dominant allelomorph had the same effect as the double dose. Complete

dominance of this kind was generally accepted in the early days of genetics as the standard for judging the relations between the phenotypes of heterozygous and homozygous individuals. All other cases were pooled under the heading of imperfect dominance.

As used in this way the concept of dominance is precise but its application is limited. The need for separating degrees of imperfect dominance gradually became felt and the standard of reference was moved to the point where dominance was absent. The degree of dominance could then be measured, and complete dominance would be merely the extreme of a continuous range of possible manifestations.

The new standard of reference, that of no dominance, introduces a new problem. At what relation between heterozygote and homozygotes can dominance be regarded as absent? It is easy to see that the heterozygotes should have a phenotype midway between those of the homozygotes, but the problem is not thereby solved, for we must ask upon what scale it should be midway. So soon as we cease to be satisfied with seeing merely that the phenotype of the heterozygote falls between those of the homozygotes, and wish to say how far it lies from each, we introduce the need for a metric and raise the question of how an appropriate scale can be devised and justified. The choice of scale can in fact determine whether we regard dominance as existing at all, and, if it does, the direction in which it is shown (Mather, 1946*a*). Suppose the character shows the levels of expression which we measure as 1 and 9 in the homozygotes, **aa** and **AA** respectively, and the level 4 in the heterozygote, **Aa**. On this scale 5 is the expression midway between the homozygotes and we must judge **a** to be partially dominant over **A**. But if for some reason we should regard a logarithmic scale as preferable, the three values become **aa** 0·00, **Aa** 0·60 and **AA** 0·95. The point midway between the homozygotes is then 0·48 and we must take the opposite view, that **A** is partially dominant. Finally, if a scale of square roots should be regarded as more suitable than either of those already considered we should have values of 1, 2, and 3, and dominance must be judged absent.

The history of interaction between genes at different loci is much the same. The idea of genic interaction was originally used by Bateson in an all-or-none fashion like that of dominance. He found cases where two allelomorphs of one gene led to different phenotypes only in the presence of particular constitutions in respect of a second gene. Complementary action, epistasis, and so on, are all variants of the same principle, differing only in the

individual dominance relations of the interacting genes and in the symmetry or lack of it in the relations between the genes. Whatever the special relations might be, interaction, as Bateson used the term, implied an absence of difference in effect between the allelomorphs of at least one of the genes under particular circumstances in respect of the other.

As with dominance there has been a shift in the standard of reference from that of complete interaction to that of no interaction. This has, of course, raised the same question, that of the metric to be used in measuring the interaction, for it is easy to see that, as with dominance, the presence, direction and degree of interaction must depend upon the scale used in representing the phenotypes which are to be compared.

A change in scale may affect the estimates of dominance and interaction in the same way. A set of genes whose effects show neither dominance nor interaction on a given scale, will appear to show both when the scale is transformed by, say, taking logarithms of the original measurements. Thus dominance and interaction may be simultaneously increased or simultaneously decreased, though not of course necessarily to equal degrees in all cases. The precise change wrought by the alteration in scale must depend on the genetical situation. Indeed, under some circumstances the change of scale may cause the measure of dominance to increase as that of interaction decreases, and *vice versa.*

Suppose that we have two genes, **A-a** and **B-b**, which in their various combinations produce the levels of expression of a character, measured on some convenient scale, as shown in Table 33*a*.

TABLE 33

(*a*)

	aa	Aa	AA
bb	1	2	3
Bb	2	4	6
BB	3	6	9

Direct Scale

No Dominance, Interaction

(*b*)

	aa	Aa	AA
bb	0·000	0·301	0·477
Bb	0·301	0·602	0·778
BB	0·477	0·778	0·954

Log Scale

Dominance, No Interaction

Neither gene shows dominance. The difference in phenotype between **Aa** and **aa** is the same as that between **AA** and **Aa** no matter what the constitution may be for **B-b**, and *vice versa.*

There is, however, interaction between the genes because the increment added when **A** is substituted for **a** is 1 in **bb**, 2 in **Bb**, and 3 in **BB** individuals. The effects of allelomorphs are additive, but those of non-allelomorphs are not.

If, however, we transform the measurements by taking logs the situation is reversed (Table 33*b*). The difference between **Aa** and **aa**, or between **Bb** and **bb** is 0·301, and that between **AA** and **Aa** or between **BB** and **bb** is 0·176, no matter what the constitution may be for the other gene. The non-additiveness is now associated only with allelomorphs. Dominance is present, but interaction is not.

Our findings with regard both to the presence of any non-additiveness in effect, and with regard to its distribution between dominance and interaction must thus be conditioned by the scale used in measuring the expression of the character. This need cause us little trouble where the genes can be followed individually. The various genotypes can then be built up at will, and their phenotypes measured on any scale or scales which seem useful. The effects of the genes in their various combinations can then be completely and unambiguously specified on any or all of the scales and a firm basis obtained for any calculation or prediction.

No such rigorous specification is possible where the genes cannot be followed and manipulated as individuals. It is then necessary to resort to biometrical analysis of the types we have been discussing, and the question immediately arises of the scale to be used. It has been shown by Fisher (1918) and Wright (1935) that the correlations between relatives may be broken down into parts ascribable to the additive effects of the genes, to their dominance and to their interaction. We have seen how variances and covariances can be broken down similarly into parts depending on the so-called additive effects (D) and on dominance (H). A third heritable component, which for purposes of discussion we may call I, depending on interaction in at least some of its forms, could have been introduced and its value estimated by an extension of the same methods. An analysis could therefore be carried out of measurements made on any given scale; but that is not to say that all scales are of equal value for this purpose.

We have already seen, in Chapter 3, that in our present state of knowledge no scale can be regarded as having a special theoretical merit. This may be expressed in another way. The relative magnitudes of D, H and I will reflect the scale used for the measurements. They will change as the scale changes, and we have no

reason to regard the values arrived at on any one scale as presenting a theoretically truer picture of the genetical situation than those yielded by any other scale. We are at liberty, therefore, to choose a scale which gives values of those components most suited to our needs.

Now the partition of variation must remain little more than an exercise in statistics unless we can put its results to some use in understanding the genetical situation in such a way as to enable us to see the consequences of inbreeding, selection or whatever other adjustment of the system we may have in mind. We seek to understand a genetical situation in order that we can foresee its consequences. The reason that it is profitable to distinguish between D, H and I is that these components depend on phenomena whose consequences differ. Generally the D component will be of most importance to us, and indeed our interest in the other components is very often confined to their elimination, for the purpose of rendering predictions based on D the more precise. At other times, however, as when considering heterosis, we may have a more direct interest in non-additive effects. But whatever our interest may be, the analysis will be the simpler the smaller the number of components there are to deal with.

The very fact that heterosis is of widespread occurrence warns us that a component of variation depending on non-additive effects of the genes must be expected, no matter what scale is used. But even so, we are still at liberty to explore the possibilities of adjusting the proportions which will appear as dominance and as interaction. Now dominance is more limited in its scope and simpler in its genetical consequences than interaction. Indeed, it is not easy to see how the consequences of all the types of interaction, which we know to occur with major genes, can be represented in as simple a way in a biometrical analysis as the separation of h from d enables us to represent dominance. Prediction is correspondingly difficult in the presence of interaction. Neither the d nor the h increments of the various genes will be additive and the correction necessitated by their non-additiveness must vary with the kind or kinds of interaction which are in operation. It is therefore preferable to have the non-additive effects represented as dominance rather than interaction, and this is what the method of scaling developed in Chapter 3 seeks to accomplish. If applied, for example, to the data of Table 33 the scaling criterion would reveal the log scale as adequate but the direct scale as inadequate for our purpose, which is of course to eliminate I, leaving only D and H as

the components of heritable variation, separable both in measurement and in prediction.

Only two of the experiments listed in Table 32 allow us to form an opinion on the success of this approach, viz. the oat and barley experiments. Should an important *I* component remain on any scale, the attempt to analyse the variation solely into *D* and *H* items should lead to inconsistencies in their values even within a generation. The test of residual interaction revealed no such inconsistencies, even though independent evidence showed that the scale was not completely adequate for prediction in the barley experiment. So far as this evidence goes, therefore, the interaction component can be neglected in suitable analyses, for on an appropriate scale interaction can either be eliminated or else its consequences can be made so small as to cause no serious disturbance in the partitioning operation. This is borne out by evidence from *Drosophila*, where Wigan (1944) and Strauss and Gowen (1943) have shown that whole chromosomes are additive in their effects on the number of sternopleural chaetae and on egg production respectively.

The oat and barley experiments also teach us something about the dominance relation. The dominance component was highly significant in the barley, and appeared large though not fully significant in the oats. In the oats the evidence indicated that the mean grain length of F_1 was midway between those of the parents. If dominance was present, therefore, it must have been balanced, some genes showing dominance of the − and others dominant of the + allelomorph. In the barley, the F_1 fell outside the limits of the parent varieties. It was, however, not so far from the mid-parent as would have been expected from the value of *H* if all the genes had shown reinforcing dominance. Again, therefore, the dominance must have been partially balanced, some of the *h* increments being in opposition to others. Now such balanced dominance could not be eliminated by re-scaling. It would appear from this evidence therefore that while interaction can be effectively eliminated by scaling, dominance cannot. We are thus led to conclude that not merely is it convenient to attempt by scaling to eliminate interaction and to throw all the non-additive effects of the genes into dominance, but further that this approach accords well with the genetical realities of the situation.

Heterosis demands some form of non-additiveness in the effects of the genes. Following Jones it has generally been assumed that this is due to dominance, and our findings support this view. It

has, however, been suggested a number of times that the relations of the allelomorphs of the genes involved may be such as to lead to super-dominance, i.e. $h > d$ so that **Aa** exceeds both **AA** and **aa**. Leaving aside for the moment the question of what can be meant by a gene which shows superdominance, it might be thought that the barley experiment lent its support to the occurrence of this phenomenon, for H was estimated in F_2 as 13169 and D as only 10389. The difference is, however, not significant. After adjustment for linkage the error variance (V) was found to be 323144 (Table 29). Now with five replications $V_{D-H} = \frac{1}{5} V(c_{DD} - 2c_{DH} + c_{HH})$ which becomes 64629(10·526+60·632+107·789). The standard error of $D-H$ is thus 3401, which is larger than the difference itself. There is therefore no reliable evidence of super-dominance in the barley, and indeed ear conformation could have shown a much greater heterosis than that observed if all the genes had possessed dominance in the same direction, without any one of them showing super-dominance (Section 21).

25 THE EFFECTIVE FACTOR

In examining the phenomena of dominance and interaction as they appear in biometrical genetics, we have seen that while we must continue to regard them as distinct in their genetical consequences, in the way made familiar by Mendelian analysis, they lose some of their distinction, even becoming partly interchangeable, in their biometrical measurement. We can no longer regard any given degree of dominance or interaction as a final property of the genes in question, because we can adjust it, or in the case of interaction eliminate it, by an artificial manipulation of the scale. Thus although the concepts derived from Mendelian genetics must remain, our notions as to the ways in which they are used must be extended and adjusted to the circumstances of biometrical analysis. In just the same way, we must still seek to explain our biometrical results in terms of the genes which Mendelian studies have led us to infer, but the new circumstances of observation make us apply the idea of genes in a modified fashion.

The fine gradation in manifestation of a character showing continuous variation suggests that the number of genes, whose differences control the variation, is large; certainly of the order of 10^1 and possibly of the order of 10^2. The values found in Table 32 for the number of effective factors are in striking contrast with this expectation. In no case does either K_1 or K_2 exceed 10, and only with the Petunias is the estimate of k itself set over this figure.

The reason for this discrepancy is, of course, clear. As we have already seen, the effective factor is not of necessity the ultimate gene.

The gene is not the only unit to which genetical investigations lead. The whole nuclear complement, the haploid set of chromosomes, the single chromosome and the chromosome segment can all behave as units in one way or another. The gene occupies its unique position solely because it is the ultimate unit, the smallest into which we are able to see the heritable material as divisible by genetic means.

Genetically we can recognize a gene only by the difference to which it gives rise. If the gene does not vary and, in varying, produce a detectable phenotypic difference, we cannot know that it is there. The inference of a gene is therefore limited by the means available for detecting the effect of its variation. If the difference produced in the phenotype is too small to be picked up by the means at our disposal we cannot identify the determinant, though we may be able to detect a group of such determinants of small effect when they are acting together.

Having detected the difference, the second condition that must be satisfied before it can be ascribed to a single gene is that it cannot be broken down into two or more smaller differences; or, amounting to the same thing, that there can be found no evidence of two or more smaller genetic differences which can combine to give the one in question. Again, since genetic differences can be followed only by their effects on the phenotype, the means of detecting these effects will limit the genetic test of divisibility.

Where two gene differences affect characters that are separately recordable, it is relatively easy to show that the genes are distinct from one another. Reassociation of the genes in the chromosomes will then be regularly detectable by the reassociation of the characters in the phenotype. The reassociation of the genes may arise by recombination or by mutation (which is not formally distinguishable from rare recombination), but no matter how rare an occurrence it may be, the reassociation of the genes can always be recognized in these circumstances as an individual event. A single recombination or mutation is therefore sufficient to establish the two genes as distinct units.

The test is less precise where the two genes are less readily distinguishable by the differences they produce in the phenotype. Suppose that the two have complementary actions such that the genotypes **aabb**, **aaB** and **Abb** give indistinguishable phenotypes,

only the combination **AB** having a different effect. On crossing two true breeding lines, **AABB** and **aabb**, the F_2 will contain only two distinguishable classes, but we may recognize that two genes are involved by the characteristic departure from a 3 : 1 ratio. If the two genes recombine freely the F_2 ratio will be 9 : 7, and there can be found types, **aaB** and **Abb**, which while phenotypically like **aabb** can be separated from it by their breeding behaviour in crosses with one another. If the two genes are linked, however, the test is less precise. The two phenotypic classes are expected in the ratio $3-2p+p^2 : 1+2p-p^2$, where p is the frequency of recombination, assumed to be the same on both male and female sides. The smaller p becomes the more difficult it will be to detect departure from the 3 : 1 ratio. Thus with $p=0{\cdot}01$, the ratio will be 2·981 : 1·019, which will be very troublesome to distinguish from 3 : 1. Furthermore, the classes **Abb** and **aaB**, which afford material for a confirmatory breeding test, will be rare. They will occur with frequencies of only $2p-p^2$ each compared with $1-2p+p^2$ for **aabb**, or 0·019 : 0·019 : 0·981 when $p=0{\cdot}01$. There is therefore no easy means of recognizing single cases of recombination between the two genes and in consequence the test is basically statistical. A unique case of recombination is insufficient: recombination must be sufficiently common for us to detect the deviations it causes from certain statistical expectations, and we cannot therefore be sure of always isolating the ultimate unit of inheritance.

Mutation might appear to help in such a case. We might obtain **aaBB** and **AAbb** by independent mutation from **AABB.** They would appear alike but on crossing would give an F_1 like the parental stock, **AABB.** A situation like this is known at the yellow locus in *Drosophila melanogaster*, but even so there is still doubt as to whether this should be regarded as two genes or one, for the mutation which would be, in our present two-gene notation, from **AB**→**ab** is more common than **AB**→**aB** or **AB**→**Ab.** The final conclusion is thus still dependent on statistical considerations. The unique observations of mutation to **aB** and **Ab** is not enough (Mather, 1946*b*).

The test of the unitary nature of a genetic difference is therefore conditioned by the type of effect on the phenotype, even with genes of major effect. This limitation of observation has its implications for the theory of the gene in general (Mather, 1946*b*); but, more germane to our present purpose, it also shows us that we can never be sure of tracing the ultimate polygene. Polygenes, by hypothesis, have effects sufficiently small by comparison with the

residual variation to prevent them being followed as individuals in genetical analysis. Their effects are also similar to one another. It is, therefore, in general impossible to detect the recombination of two polygenes as a unique event by its effect in the phenotype. The test of recombination must be statistical.

There are two reasons why the test of recombination between polygenes will be even less sensitive than the test which we have considered in the case of linked complementary genes. The first reason is that recombination must be sufficiently frequent for us to detect a change in variance depending at best (Section 16) on the difference between $(1-2p)$ and $(1-2p)(1-p)$. This change is from 0·9800 to 0·9702 when $p=0{\cdot}01$ and is only from 0·80 to 0·72 when p is as high as 0·1. Secondly, we have no means of separating the effects on variation of all the pairs of genes which are segregating, so that change in some of them may be partly swamped by lack of it in others. The extent to which we can push the analysis of a polygenic system into the ultimate units which genetics has taught us to recognize, must thus be limited by the conditions under which our observations are made. The less the extraneous variation relative to the effect of the gene, and the fewer the genes which are segregating, the further the analysis can be taken. But in the general case, while we must interpret the properties of a polygenic system in terms of ultimate genes, we must be prepared to use biometrical units specified by analytical criteria different from those of Mendelian genetics, and depending on statistical relations rather than unique events.

Investigations using major genes have given us a picture of the chromosomes (satisfactory for most purposes of predicting behaviour in hereditary transmission though admittedly unsatisfactory in other ways) as composed of units, the genes, within which recombination does not occur but between which it does occur. When we cannot recognize recombination as an individual event, we must of necessity modify this picture to one in which recombination occurs with a given frequency between neighbouring units, while it still does not occur inside a single unit. Now, no matter how low we set this critical recombination frequency, we can still have no certainty that the factors so distinguished will show no internal recombination. We are in fact assuming a discontinuity which may be spurious in the distribution of recombination along the chromosome.

The number of factor into which a chromosome can be divided in this way cannot, of course, exceed the ratio its total genetic length

bears to the recombination frequency assumed between adjacent factors. Since we may assume that factors in different chromosomes recombine freely, their numbers are additive over chromosomes. The maximum number of factors we can find is thus dependent on the assumption made as to the frequency of recombination between them. If we assume that the factors are independent in inheritance, statistically the easiest assumption to make (see Chapter 6), the maximum number is given by the recombination index, i.e. the haploid number of chromosomes plus the mean number of chiasmata per nucleus.

This assumption of independent inheritance is equivalent to supposing that there is no variation in either the number of chiasmata formed or in the position in which each forms within the chromosome, at least in respect of chiasmata falling in parts of the chromosomes carrying genetic differences. In such a case pairs of genes will either recombine freely or will not recombine at all, according to whether they do or do not lie astride a point of chiasma formation. The factor is then clearly the same as the gene which could be detected by Mendelian methods, since chiasma formation, upon which recombination within a chromosome depends, is either all or none. This situation, or something closely approximating to it, is encountered in such species as *Mecostethus grossus* and *Fritillaria meleagris* where the chiasmata can be observed to be localized in position. In heterokaryotic fungi, too, where no recombination occurs within a nucleus and the variation arises from the sorting out of whole nuclei, recombination is all or none. The nucleus is then the final unit of recombination and transmission, as is the segment delimited by the positions of chiasma formation in *Mecostethus* and *Fritillaria*. It is at once the gene and the effective factor. The latter will therefore be as constant a unit as the gene in these cases.

Generally, however, chiasmata vary in their position of formation. The linkage test of Chapter 5 is in fact a test of such variation. If all pairs of genes either recombined freely or not at all, they would contribute either as two independent units or only as one to D and H. There could then be no change in D and H over the generations. Such changes have been clearly observed in barley and they are strongly suggested in both *Drosophila* and *Antirrhinum*. In *Petunia* the linkage was inferred on other grounds, and the maize data are insensitive. Only in the oats did linkage fail to appear in a test of reasonable sensitivity. Even in such a case the failure to observe the type of change in D and H, which recom-

bination values between 50% and 0% bring about, may only mean that the genes were so distributed as not to offer the means of detecting the linkage. However this may be, the linkage test offers us a means of deciding whether we may safely assume the factors to be inherited independently. When linkage is detected we cannot fairly make this assumption, and any count of the number of factors based on independence must, as we saw with the barley, be too low.

The linkage test has a still further importance. We need not assume our factors to recombine freely. As with the barley we may assume other frequencies of recombination which suit our convenience, and obtain useful predictions from them. But whereas if no linkage is found, we have grounds for assuming that our factors, based on the assumption of independence, will be reasonably stable for reasons which we have just discussed, the factors cannot be stable which we arrive at by assuming recombination values of other than 50% when linkage has been demonstrated by change in D and H. The assumption that factors are separated by, say, 10% recombination is equivalent to supposing that a chiasma, while not forming regularly in one given position, must form in one or other of only 10 positions, and never fall between them. This may be a useful assumption for purposes of immediate calculation, but it is obviously fictitious. The factors arrived at in this way must be in a sense statistical abstractions. Each will vary in its genetic content within a generation and will be broken down by further recombination as the generations succeed one another. The demonstration of linkage carries with it the demonstration that no permanent system of factors can be derived.

The situation may be explored further by determining the distribution of the number of factors among F_3's, using F_4 data for the purpose. If the factors presented in F_2 were stable, each would have a half chance of segregating in any F_3. This enables us to formulate an expectation for the distribution of any given number of factors observed in F_2 among the F_3 families, and the numbers observed can be compared with this expectation. The discrepancies will tell us something of how our factors differ and change in the magnitudes of their action and thus of how they are being broken down by recombination.

This breakdown of the effective factors by recombination has three consequences. First of all, the number of factors must appear to increase as the experiment progresses. Thus with 4 factors segregating in F_2 and no change by recombination within them, F_3

families should average 2 segregating factors, F_4's 1 segregating factor, and so on. But with breakdown, the fall in number per family in the succeeding generations will be less, so that the number which must be assumed in the experiment as a whole will increase. At the same time, of course, the average effect of each factor must diminish correspondingly. In other words, the gap between gene and effective factor will be narrowing as the experiment proceeds, and if this process is continued for sufficiently long we might hope that the biometrical factor would ultimately approximate to the individual polygene. The experiment would, however, need to be very lengthy for individual polygenes to be counted in this way.

A second consequence of the breakdown of the factors is that they can be used as units only in a temporary sense. They enable us to foresee behaviour in the near future, to predict minimal limits and rates of advance under selection for one or two generations. Where the linkage test reveals little evidence of change in the units, the period over which prediction can be made may be increased; but even in this case, the longer the term of the prediction the more hazardous it must be. The number of effective factors must therefore be used with reserve: unlike genes, they are not final units.

Nevertheless, it is worth noting that the factors are the only units we have. We can arrive at some estimate of their number; whereas we can arrive at no estimate of the number of genes, except possibly with great labour. And even could we know how many genes were involved the information would benefit us little, for their organization within the chromosomes is all-important in determining the contributions they make to the components of variability and to the selective responses. It is of more use to us to consider the system in terms of contributions to the variability than in terms of individual genes. The factors to which the analysis of variability leads us are admittedly neither permanent nor constant, so that the predictions which they permit have only a short range validity; but a knowledge of the number of genes without any knowledge of their organization into factors would permit no prediction at all.

The third consequence of recombination within the effective units is that just as the unit is broken down in this way, it can also be built up. Genes themselves change only by the rare process which we term mutation, and we know that polygenes are no exception (Mather, 1941; Mather and Wigan, 1942). Recombination

on the other hand is much more frequent in its occurrence, and it therefore offers much greater possibility of useful change in the factor. The effects, however, of intra-genic mutation and inter-genic recombination will not be directly distinguishable where the genes have physiologically small, similar and supplementary actions. We can recognize change through recombination within the factor only by comparison of the speed of the change observed with that known to be due directly to mutation as measured in an inbred line (Mather, 1941). From the point of view of the effective factor, viewed as a whole, the two processes are therefore inseparable.

The effects of recombination in slowly building up linked groups of polygenes which will tend to stick together in segregation and so appear more or less as one large factor, in the way we have been discussing, is well shown by the selection experiment with *Drosophila* described in Section 2 (Mather and Harrison, 1948). The first selection line advanced slowly and fairly steadily for 20 generations, the mean chaeta number increasing by about 20 over that period (Fig. 4). Such a change is too slow to be wholly, or even largely, due to recombination of whole chromosomes: there are only three large chromosomes in *Drosophila melanogaster*, and all their combinations as whole chromosomes would appear and be exposed to selection in the first few generations. The main advance must have been due rather to recombination within chromosomes, a number of repulsion linkages, each of the kind $\frac{+-}{-+}$ being progressively broken down and replaced by coupling linkages of the kind ++.

This recombination also upset the balance of other systems of polygenes one of which affected fertility. As we saw in Section 2, fertility fell as chaeta number rose, until finally it became so low and the flies so few that selection could no longer be practised and mass cultures had to be used. With selection relaxed for chaeta number, the fertility system took charge and as fertility rose under natural section, chaeta number fell. But it fell at such a speed that 80% of the gain in chaeta number, which took 20 generations to achieve, was lost in only 4 more. When selection was again practised for increased chaeta number, the lost ground was regained once more in 4 generations. The coupling linkages so laboriously built up, had persisted with corresponding tenacity, so that the original segregation for factors which, by reason of their balanced genic content, were of small effect was

replaced by segregation for factors of larger effect. The original advance had been conditioned by the slow building up of the very polygenic combinations whose more or less unitary behaviour in transmission and segregation permitted later advance at four times the original speed.

Thus in this experiment, as indeed must be the case in all such experiments, the speed and immediate limit of advance under selection was not determined by the number of individual polygenes but by their organization into linked combinations. An experiment covering only a few generations will therefore discover the properties of these combinations rather than those of their constituents. In consequence the factors, in terms of which we interpret biometrical experiments, must be related to the combinations rather than to the individual genes. The individual polygene must remain, however, as the final unit of action, change and recombination upon which the properties of the combinations or factors are based. We are in fact working at two levels. In the immediate sense the combination is all important, but it is not a final unit. Genically it is composite and it can therefore change as its constituent genes change or become reassociated.

This distinction between the gene and the effective factor, which we recognize in biometrical segregation, is important to our understanding and use of the latter concept. The factor must derive its properties from the genes of which it consists, but these properties need not be so limited as those of a gene. We have seen that change in a factor depends more on the reassociation of the constituent genes than on change within the genes themselves. We must now observe that we cannot even be sure that a change which is not to be traced to recombination is due to mutation of one gene. Structural change, leading, for example, to duplication of several genes within a factor, cannot be distinguishable from single gene mutation, and indeed the apparent mutations of Jones (1944) in inbred maize may well be of this kind.

In the same way, super-dominance ($h > d$) of an effective factor, should it be observed, could always be interpreted in terms of normal dominance ($h \leqslant d$) of individual genes of like action which were linked, perhaps very closely, in the repulsion phase. Equally, too, apparent pleiotropy of the effective factor could always be interpreted as due to its containing within the chromosome segment which is its physical basis, polygenes belonging to unlike systems. Genetical experiments have in fact shown that genes of unlike effect are characteristically mingled along the chromosomes.

Apparent pleiotropic action, due really to linked genes, must therefore be expected, and the observations on correlated responses to selection (Section 2) vindicate this expectation. These correlated responses may in fact be very complex. In the selection experiment in question, fertility, spermatheca number and mating behaviour all changed as a result of selection for chaeta number. Doubtless other characters not recorded in the experiment behaved similarly. Pleiotropy in the classical sense is therefore almost useless as a concept for application in biometrical genetics. We know that apparent pleiotropy can be due to linkage, and we know that units of inheritance are linked groups of genes. We have, and indeed we can have, no proven case of pleiotropy of a single polygene.

In order to account for continuous variation we have had to postulate systems of genes having small, similar and supplementary effects. We have been able to show that these genes must behave in transmission in the same way that the major genes of Mendelian genetics behave, and on this basis we have been able to devise a means of analysing continuous variation. But we have also come to see that, in biometrical genetics, observations can be made only on linked groups of the genes, on in fact the total genic content of factors whose physical basis is to be found in whole segments of the chromosomes. The properties of the groups or factors will reflect not merely the behaviour of each individual constituent gene, but also the mechanical and physiological relations of the genes one with another. And since we have no means of predicting these genic relations in detail, we can learn the properties of the factors only by direct observation and experiment.

Something of the properties of integration within the factors has already been discovered, sufficient at least to see the situation which faces us. The necessary analytical methods are also taking shape. By the future application and refinement of these methods we should reach a fuller understanding of the units of biometrical inheritance, and thereby gain a greater control over their practical use.

GLOSSARY OF SYMBOLS

1. GENETICAL SYMBOLS

A-a; **B-b** &c.,	genes of two allelomorphs; **A**, **B**, &c., being the allelomorphs which add to the expression of the character
P_1 and P_2	the two parents of a cross
F_1	the first generation of the cross, from $P_1 \times P_2$ or $P_2 \times P_1$
F_2	the second generation of the cross, from F_1 individuals self-fertilized or interbred
F_3	the third generation of the cross. from F_2 individuals self-fertilized
BIP	(biparental progenies) the third generation of the cross, from F_2 individuals interbred in pairs taken at random
MAT	(maternal progenies) the third generation of the cross, from F_2 individuals allowed to breed as females at random with the rest of the F_2
1B	the first backcross generation, from crossing F_1 to the parents; consisting of B_1 from $F_1 \times P_1$ or reciprocally and B_2 from $F_1 \times P_2$ or reciprocally
2B	the second backcross generation, from crossing 1B to the parents; consisting of B_{11} from $B_1 \times P_1$ or reciprocally B_{12} from $B_1 \times P_2$ or reciprocally B_{21} from $B_2 \times P_1$ or reciprocally and B_{22} from $B_2 \times P_2$ or reciprocally
u_a	the frequency of allelomorph **A** in the gametes of a randomly breeding population
v_a	the frequency of allelomorph **a** in the gametes of a randomly breeding population $(=1-u_a)$
p_{ab}	the frequency of recombination between the genes **A-a** and **B-b**. $q_{ab}=1-p_{ab}$

2. GENERAL SYMBOLS

d_a	the increment added to (or subtracted from) the measurement by $\underline{\mathbf{AA}}$ (or **aa**) Thus $\overline{\mathbf{AA}}-\overline{\mathbf{aa}}=2d_a$
h_a	the increment added to the measurement by **Aa** Thus $\overline{\mathbf{Aa}}-\frac{1}{2}\overline{\mathbf{AA}}+\overline{\mathbf{aa}}=h_a$
D	the component of variation arising from the d increments of all the genes which are segregating
H	the component of variation arising from the h increments of all the genes which are segregating
E	the component of variation arising from non-heritable agencies. It appears as E_1 the non-heritable variation of individuals E_2 the non-heritable variation of means values of families and any other special values, suitably denoted, which it may be necessary to estimate

$_HV$	the heritable portion of the variance, including contributions from both D and H
$_EV$	the non-heritable portion of the variance
k	the number of effective factors
K_1	the estimate of k found as $\frac{(\bar{P}_1-\bar{P}_2)^2}{4D}$
K_2	the estimate of k found as $\frac{_H\bar{V}^2_{F3}}{V_{VF3}}$ where $_H\bar{V}_{F3}=\frac{1}{4}D+\frac{1}{8}H$ and V_{VF3} is corrected for sampling variation
V_α	the variance of d_a, d_b, &c., round their mean d
V_β	the variance of $(d_a^2+\frac{1}{2}h_a^2)$, $(d_b^2+\frac{1}{2}h_b^2)$, &c., round their mean
r	the ratio of V_β to V_α

3. STATISTICAL SYMBOLS

n	the number of individuals measured
$\bar{x}$	the mean of the values of variate x, found as $\frac{S(x)}{n}$
V_x	the variance of the values of x round $\bar{x}$, found as $\frac{1}{n-1}S(x-\bar{x})^2=\frac{1}{n-1}\left[S(x^2)-\frac{S^2(x)}{n}\right]$
$V_{\bar{x}}$	the variance of the $\bar{x}$, found as $\frac{V_x}{n}$ for single means, or by direct estimation where several means are available
s_x	the standard deviation of the distribution of x, found as $\sqrt{V_x}$
$s_{\bar{x}}$	the standard error of $\bar{x}$, found as $\sqrt{V_{\bar{x}}}$
$W_{x/y}$	the covariance of variates x and y round their respective means, found as $\frac{1}{n-1}S[(x-\bar{x})(y-\bar{y})]=\frac{1}{n-1}S[x(y-\bar{y})]=\frac{1}{n-1}\left[S(xy)-\frac{S(x)S(y)}{n}\right]$

4. SPECIAL STATISTICAL SYMBOLS

$\bar{P}_1$, $\bar{P}_2$, $\bar{F}_1$, $\bar{F}_2$, $\bar{B}_1$, etc., the mean values of the measurement in the families P_1, P_2, F_1, F_2, B_1, &c., respectively

V_{P1}, V_{P2}, V_{F1}, V_{F2}, V_{B1}, V_{B2}, the variances of the measurement in the families P_1, P_2, F_1, F_2, B_1, B_2 respectively

$V_{\bar{F}3}$, $V_{\bar{BIP}}$, $V_{\bar{B}11}$, $V_{\bar{B}12}$, $V_{\bar{B}21}$, $V_{\bar{B}22}$, the variance of the mean measurements of all the families in F_3, BIP, B_{11}, B_{12}, B_{21}, B_{22} respectively

$\bar{V}_{F3}$, $\bar{V}_{BIP}$, $\bar{V}_{B11}$, $\bar{V}_{B12}$, $\bar{V}_{B21}$, $\bar{V}_{B22}$, the mean variance of all the families in F_3, BIP, B_{11}, B_{12}, B_{21}, B_{22} respectively

V_{VF3}, the variance of the variances of all the families in F_3

$W_{F2/F3}$, $W_{F2/BIP}$, the covariances of the measurements of F_2 individuals with the means of their F_3 and biparental progenies respectively

$W_{B1/B11}$, $W_{B1/B12}$, $W_{B2/B21}$, $W_{B2/B22}$, the covariances of the measurements of B_1 and B_2 individuals with the means of their B_{11}, B_{12}, B_{21} and B_{22} progenies respectively

$W_{P/O}$ the covariance of parent and offspring in a randomly breeding population

$W_{S/S}$ the covariance of full siblings in a randomly breeding population

$r_{P/O}$ the correlation of parent and offspring in a randomly breeding population

$r_{O/O}$ the correlation between full siblings in a randomly breeding population

5. SCALING TESTS

$$A = 2\bar{B}_1 - \bar{F}_1 - \bar{P}_1, \qquad V_A = 4V_{\bar{B}_1} + V_{\bar{F}_1} + V_{\bar{P}_1}$$
$$B = 2\bar{B}_2 - \bar{F}_1 - \bar{P}_2, \qquad V_B = 4V_{\bar{B}_2} + V_{\bar{F}_1} + V_{\bar{P}_2}$$
$$C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2, \qquad V_C = 16V_{\bar{F}_2} + 4V_{\bar{F}_1} + V_{\bar{P}_1} + V_{\bar{P}_2}$$
$$A = B = C = 0 \text{ on an adequate scale}$$

REFERENCES

BATESON, W. (1909) *Mendel's Principles of Heredity.* University Press' Cambridge

BEADLE, G. W. (1945) Biochemical Genetics. *Chem. Rev.*, **37**, 15–96

CHAPMAN, A. B. (1946) Genetic and non-genetic sources of variation in the weight response of the immature rat ovary to a gonadotrophic hormone. *Genetics*, **31**, 494–507

CHARLES, D. R., and GOODWIN, R. H. (1943) An estimate of the minimum number of genes differentiating two species of golden-rod with respect to their morphological characters. *Amer. Nat.*, **77**, 53–69

CHARLES, D. R., and SMITH, H. H. (1939). Distinguishing between two types of gene action in quantitative inheritance. *Genetics*, **24**, 34–48

CULBERTSON, J. O. (1942) Inheritance of factors influencing sucrose percentage in *Beta vulgaris*. *J. Agr. Res.*, **64**, 153–71

DARLINGTON, C. D. (1939) *Evolution of Genetic Systems.* University Press, Cambridge

DARLINGTON, C. D., and LA COUR, L. F. (1941) The detection of inert genes. *J. Hered.*, **32**, 115–21

DARLINGTON, C. D., and MATHER, K. (1948) *The Elements of Genetics.* Allen and Unwin, London

DARLINGTON, C. D., and THOMAS, P. T. (1941) Morbid mitosis and the activity of inert chromosomes in *Sorghum*. *Proc. Roy. Soc. B.*, **130**, 127–50

EAST, E. M. (1915) Studies on size inheritance in *Nicotiana*. *Genetics*, **1**, 164–76

EMERSON, R. A., and EAST, E. M. (1913) The inheritance of quantitative characters in maize. *Bull. Agric. Exp. Sta., Nebraska.* Res. Bull. 2

FISHER, R. A. (1918) The correlation between relatives on the supposition of Mendelian inheritance. *Trans. Roy. Soc. Edin.*, **52**, 399–433

FISHER, R. A. (1930) *The Genetical Theory of Natural Selection.* Clarendon Press, Oxford

FISHER, R. A. (1936) The use of multiple measurements in taxonomic problems. *Ann. Eugenics*, **7**, 179–88

FISHER, R. A. (1938) The statistical use of multiple measurements. *Ann. Eugenics*, **8**, 376–86

FISHER, R. A. (1946) *Statistical Methods for Research Workers.* 10th Edn. Oliver and Boyd, Edinburgh

FISHER, R. A., IMMER, F. R., and TEDIN, O. (1932) The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. *Genetics*, **17**, 107–24

FLORELL, V. (1924) Studies on the inheritance of earliness in wheat. *J. Agr. Res.*, **29**, 333–47

FREEMAN, G. F. (1919) The heredity of quantitative characters in wheat. *Genetics*, **4**, 1–93

GALTON, F. (1889) *Natural Inheritance.* Macmillan, London

GOODWIN, R. H. (1944) The inheritance of flowering time in a short-day species, *Solidago sempervirens* L. *Genetics*, **29**, 503–19

GRÜNEBERG, H. (1938) An analysis of the 'pleiotropic' effects of a new lethal mutation in the rat (*Mus norvegicus*). *Proc. Roy. Soc. B.*, **125**, 123–43

HARLAND, S. C. (1936) The genetical conception of the species. *Biol. Revs.*, **11**, 83–112

HOSHINO, Y. (1915) On the inheritance of flowering time in peas and rice. *J. Coll. Agric. Tohoku Imp. Univ. Sapporo*, **6**, 229–88

JOHANNSEN, W. (1909) *Elemente der exakten Erblichkeitslehre.* Fischer, Jena

JONES, D. F. (1945) Heterosis resulting from degenerative changes. *Genetics*, **30**, 527–42

LAWRENCE, W. J. C., and SCOTT-MONCRIEFF, R. (1935) The genetics and chemistry of flower colour in *Dahlia*: a new theory of specific pigmentation. *J. Genet.*, **30**, 155–226

LEAKE, H. M. (1911) Studies in Indian cotton. *J. Genet*, **1**, 205–72

LUSH, J. L. (1943) *Animal Breeding Plans.* 2nd Edn. Iowa State College Press, Ames

MACARTHUR, J. W., and BUTLER, L. (1938) Size inheritance and geometric growth processes in the tomato fruit. *Genetics*, **23**, 253–68

MATHER, K. (1941) Variation and selection of polygenic characters. *J. Genet.*, **41**, 159–93

MATHER, K. (1942) The balance of polygenic combinations. *J. Genet.*, **43**, 309–36

MATHER, K. (1943*a*) Polygenic inheritance and natural selection. *Biol. Revs.*, **18**, 32–64

MATHER, K. (1943*b*) *Statistical Analysis in Biology.* Methuen, London.

MATHER, K. (1944) The genetical activity of heterochromatin. *Proc. Roy. Soc. B.*, **132**, 308–32

MATHER, K. (1946*a*) Dominance and heterosis. *Amer. Nat.*, **80**, 91–6

MATHER, K. (1946*b*) Genes. *Sci. J.R. Coll. Sci.*, **16**, 63–71

MATHER, K., and BARTON-WRIGHT, E. C. (1946) Nicotinic acid in sugary and starchy maize. *Nature*, **157**, 109

MATHER, K., and EDWARDS, P. M. J. (1943) Specific differences in *Petunia*—III. Flower colour and genetic isolation. *J. Genet.*, **45**, 243–60

MATHER, K., and HARRISON, B. J. (1948) The manifold effect of selection. *Heredity.* (In the press)

MATHER, K., and WIGAN, L. G. (1942) The selection of invisible mutations. *Proc. Roy. Soc. B.*, **131**, 50–64

MENDEL, G. (1865) Experiments in plant hybridization. Translation in Bateson (1909)

NILSSON-EHLE, H. (1909) *Kreuzungsuntersuchungen an Hafer und Weizen.* Lund

ÖSTERGREN, G. (1947) Heterochromatic B-chromosomes in *Anthoxanthum. Hereditas*, **33**, 261–96

PANSE, V. G. (1940*a*) The application of genetics to plant breeding—II. The inheritance of quantitative characters and plant breeding. *J. Genet.*, **40**, 283–302

PANSE, V. G. (1940*b*) A statistical study of quantitative inheritance. *Ann. Eugen.*, **10**, 76–105

POWERS, L. (1941) Inheritance of quantitative characters in crosses involving two species of *Lycopersicon*. *J. Agr. Res.*, **63**, 149–74

POWERS, L. (1942) The nature of the series of environmental variances and the estimation of the genetic variances and the geometric means in crosses involving species of *Lycopersicon*. *Genetics*, **27**, 561–75

QUISENBERRY, K. S. (1926) Correlated inheritance of quantitative and qualitative characters in oats. *Bull. Agric. Exp. Sta., West Virginia*, 202

RASMUSSON, J. M. (1933) A contribution to the theory of quantitative character inheritance. *Hereditas*, **18**, 245–61

RASMUSSON, J. M. (1935) Studies on the inheritance of quantitative characters in *Pisum*—I. Preliminary note on the genetics of flowering. *Hereditas*, **20**, 161–80

SAX, K. (1923) The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics*, **8**, 552–60

SEREBROVSKY, A. S. (1928) An analysis of the inheritance of quantitative transgressive characters. *Z.I.A.V.*, **48**, 229–43

SMITH, H. F. (1936) A discriminant function for plant selection. *Ann. Eugenics*, **7**, 240–50

SMITH, H. H. (1937) The relations between genes affecting size and colour in certain species of *Nicotiana*. *Genetics*, **22**, 361–75

STRAUSS, F. S., and GOWEN, J. (1943) Heterosis: its mechanism in terms of chromosome units in egg production of *Drosophila melanogaster*. *Genetics*, **28**, 93

'STUDENT' (1934) A calculation of the minimum number of genes in Winter's selection experiment. *Ann. Eugenics*, **6**, 77–82

SVARDSON, G. (1944) Polygonic inheritance in *Lebistes*. *Arkiv. f. Zool.*, **36A**, 1–9

THOMPSON, W. P. (1918) The inheritance of the length of the flowering and ripening periods in wheat. *Trans. Roy. Soc. Can.* (Ser. 3), **12**, 69–87

WARREN, D. C. (1924) Inheritance of egg size in *Drosophila melanogaster*. *Genetics*, **9**, 41–69

WIGAN, L. G. (1944) Balance and potence in natural populations. *J. Genet.*, **46**, 150–60

WIGAN, L. G. (1948) Distribution of polygenic activity in the X chromosome of *Drosophila melanogaster*. (In preparation)

WIGAN, L. G., and MATHER, K. (1942) Correlated response to the selection of polygenic characters. *Ann. Eugenics*, **11**, 354–64

WRIGHT, S. (1934*a*) An analysis of variability in number of digits in an inbred strain of guinea pigs. *Genetics*, **19**, 506–36

WRIGHT, S. (1934*b*) The results of crosses between inbred strains of guinea pigs, differing in number of digits. *Genetics*, **19**, 537–51

WRIGHT, S. (1935) The analysis of variance and the correlation between relatives with respect to deviations from an optimum. *J. Genet.*, **30**, 243–56